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Chromosome Abnormalities and Genetic Counseling (4 ed.)

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Elements of Medical Cytogenetics

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CHROMOSOMES WERE first seen and named in the late nineteenth century. *Chromosome* is a combination of Greek words meaning colored (*chrom*) body (*soma*); the word was coined by the illustrious German anatomist Heinrich Wilhelm Gottfried von Waldeyer-Hartz. It was early appreciated that these brightly staining objects appearing in the cell nucleus must be the "stuff of heredity," the very vessels of our genetic inheritance. Most observers had concluded, in the earlier part of the twentieth century, that the human chromosome count was 48. It was not until the 1950s, due to technical advances, and in particular the use of a hypotonic solution to swell the cells, giving an uncluttered view of the chromosomes, that Joe Hin Tjio and Albert Levan could recognize that 46 was the correct number. This discovery spurred research into conditions in which a chromosomal cause had hitherto been suspected; and in 1959 ("the wonderful year of human cytogenetics") came the first demonstrations of a medical application of the new knowledge, with practically simultaneous discoveries of the chromosomal basis of Down syndrome, Klinefelter syndrome, and of Turner syndrome (Lejeune et al., [1] 1959; Jacobs and Strong, 1959; Ford et al., 1959), and these were followed shortly thereafter by the recognition of the other major aneuploidy syndromes. Harper (2006) records the history, and the personalities behind the history, in his book *First Years of Human Chromosomes*; a book that should be read by every student of medical cytogenetics with an interest in how their discipline came to be. Harper points out that the practice of genetic counseling came into its own essentially upon the basis of these chromosomal discoveries: so to speak, geneticists now had "their organ."

"Colored bodies" became an especially apt derivation with the development of various different staining techniques in the 1980s and 1990s, showing different parts of chromosomes in many different colors, whether true or computer-generated false colors. The images produced by this kaleidoscopic karyotyping could be rather beautiful. Black-and-white photographs are less splendid but often suffice (Fig. **1–1**). Albeit that molecular methodologies are now taking over from classical cytogenetics, and providing a different view of the genetic material, the word *chromosome* will surely last forever.

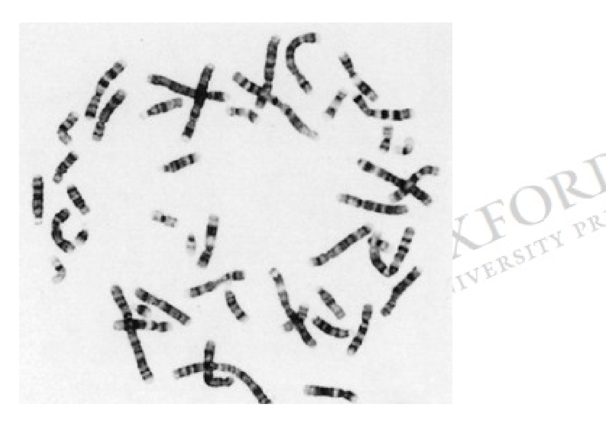




Figure 1–1
Banded chromosomes as they appear viewed through the microscope.

Chromosomal Morphology

Chromosomes have a linear appearance: two arms that are continuous at the *centromere*. Reflecting the French influence in the establishment of the cytogenetic nomenclature, the shorter arm is designated *p* (for petit), and the longer is *q* (variously explained as being the next letter in the alphabet, a mistyping of g (for grand), or as the other letter in the formula p + q = 1). In the early part of the cell cycle, each chromosome is present as a single structure, a chromatid, a single DNA molecule. During the cell cycle (Fig. **1–2**) the chromosomes replicate, and two *sister chromatids* form. Now the chromosome exists as a double-chromatid entity. Each chromatid contains exactly the same genetic material. This replication is in preparation for cell division so that, after the chromosome has separated into its two component chromatids, each daughter cell receives the full amount of genetic material. It is during mitosis that the chromosomes contract and become readily distinguishable on light microscopy. (At other times in the cell cycle, chromosomes are attenuated and not visible as such.)













Figure 1–2
Chromosome replication and separation during the mitotic cycle.

Routine classical cytogenetic analysis is done on mitotic cells, usually obtained from blood. Blood lymphocytes have two convenient properties for the cytogeneticist: they are easily obtained, and they are easily stimulated to go into mitosis. The chromosomes of the small number of lymphocytes studied are taken as representative of the chromosomal constitution of (essentially) every other cell of the body. Blood (specifically, nucleated white cells) is also the tissue from which DNA is extracted in routine microarray analysis. In the case of prenatal diagnosis, the cells from amniotic fluid or chorionic villi are the source material for both karyotyping and microarray analysis; these tissues are assumed (with certain caveats) to represent the fetal chromosomal constitution.

The 46 chromosomes come in 23 matching pairs and constitute the *genome*. One of each pair came from the mother, and one from the father. For 22 of the chromosome pairs, each member (each *homolog*) has the same morphology in each sex: these are the *autosomes*. The *sex chromosome* (or *gonosome*) constitution differs: the female has a pair of X chromosomes, and the male has an X and a Y chromosome. The single set of homologs—one of each autosome plus one sex chromosome—is the haploid set. The *haploid* number (*n*) is 23. The haploid complement exists, as such, only in the gametocytes (ovum and sperm). All other cells in the body—the *soma*—have a double set: the diploid complement (*2n*) of 46. If there is a difference between a pair of homologs, in the sense of one being structurally rearranged, the person is described as a *heterozygote*.

The chromosomes are classically distinguishable on the basis of their size, centromere position, and banding pattern. The centromere may be in the middle, off-center, or close to one end—metacentric, submetacentric, and acrocentric, respectively. The chromosomes are numbered 1 through 22, and X and Y, and are also assigned to groups A through G, according to their general size and the position of the centromere. The diagrammatic representation of the banding pattern is the *ideogram* (Appendix A). The numbering is based on size, largest to smallest (to split hairs, this order is not exact; for example, chromosomes 10 and 11 are shorter than chromosome 12, and chromosome 21 is smaller than 22). Certain parts of some chromosomes may show variation (*heteromorphism*) in the population. Increasing precision in banding permitted progressively more subtle definition of the chromosome (Fig. **1–3**); microarrays take this to a further level.



Figure 1–3
Increasing resolution of banding (chromosome 11). (Courtesy D. R. Romain.)

Chromosomes are conventionally displayed cut out from a photograph or captured from an electronic image, and arranged as a "paste-up," with p arms upward, in their matching pairs. This paired-up presentation is called a *karyotype*, or *karyogram* (Fig. **1–4**); the word *karyotype* is also used in the general sense of "chromosomal constitution." Those coming from a DNA-based view may see the chromosome lying on its side, and microarray reports usually show a horizontal depiction of the chromosome arms, with the graph indicating duplications and deletions by a rise or a fall compared to baseline, respectively (although no one is proposing that short and long arms be renamed as left and right!). Karyotypes are described according to a shorthand notation, the International System of Human Cytogenetic Nomenclature (ISCN, 2009); an outline is given in Appendix B.

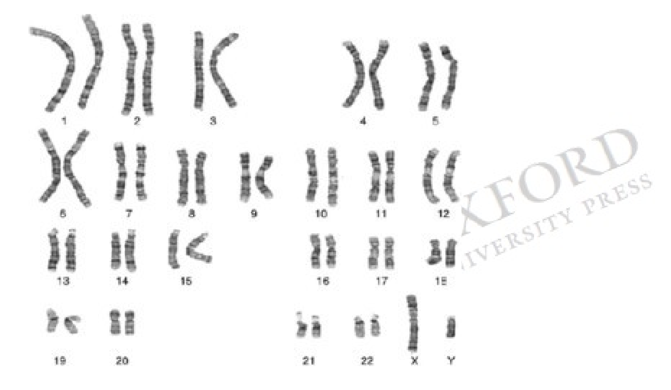


Figure 1–4
Chromosomes arranged in formal karyotype.



Chromosomal Structure and Function

The two chemical components of chromatin are DNA and protein. Some of the stains used to produce chromosome banding patterns stain the DNA, while others stain the proteins. Proteins provide the scaffolding of the chromosome and are divided into histone and non-histone proteins. Histones are strongly conserved DNA-associated proteins; the fact that they differ little between species such as ourselves and the sweet pea (for example) indicates how fundamentally important is their role in maintaining the integrity of the chromosome.

Chromatin exists in differently condensed forms: the less condensed *euchromatin* and the more condensed *heterochromatin*. Euchromatin contains the coding DNA—the genes —while heterochromatin comprises noncoding DNA. Chromosomes are capped at the terminal extremities of their long and short arms by *telomeres*, specialized DNA sequences comprising many repeats of the sequence TTAGGG, that can be thought of as sealing the chromatin and preventing its fusion with the chromatin of other chromosomes. The *centromere* is a specialized region of DNA that, at mitosis, provides the site at which the spindle apparatus can be anchored and draw each separated chromatid to opposite poles of the dividing cell. Centromeric heterochromatin contains "satellite DNA," so-called because these DNA species have different buoyant densities and produce distinct humps on a density gradient distribution. (These are not to be confused with the satellites on acrocentric chromosomes.) A separate issue is the "packaging question": how the centimeters of DNA are compacted into micron-length chromosomes. The presently preferred model is that the chromatin fibers are thrown into loops extending outward from a backbone, this backbone being formed as adhesive sites dotted along the fibers come together and construct a continuous linear stack. Miller

and Therman (2001) treat this question in detail, and Annunziato (2008) provides a popular source.

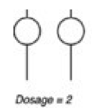
Chromosome Abnormality

Chromosomes are distributed to each daughter cell during cell division in a very precise process—precise, but prone to error. From our perspective, the two cell divisions of *meiosis*, during which the gametes are formed, are of central importance. Most of the discipline of medical cytogenetics focuses on the consequences of disordered meiosis having produced a chromosomally abnormal gamete, causing a chromosomal abnormality in the *conceptus*. A chromosome abnormality that is present from conception and involves the entire body is a *constitutional* abnormality. If an additional cell line with a different chromosomal complement arises before the basis of the body structure is formed (i.e., in embryonic or pre-embryonic life) and becomes an integral part of the organism, *constitutional mosaicism* results. In this book, we concern ourselves practically solely with constitutional abnormalities. Acquired chromosomal abnormality of course exists, and indeed it is a major initiating and sustaining cause in most cancers, a fact first proposed by Boveri in 1914, and voluminously attested by the work of Mitelman et al. (2011); but this is more the field of study of the molecular oncologist than the genetic counselor.

An incorrect amount of genetic material carried by the conceptus disturbs and distorts its normal growth pattern (from zygote → blastocyst → embryo → fetus). In *trisomy* there is three of a particular chromosome, instead of the normal two. In *monosomy* only one member of the pair is present. Two of each is the only combination that works properly! It is scarcely surprising that a process as exquisitely complex as the development of the human form should be vulnerable to a confused outflow of genetic instruction from a nucleus with a redundant or incomplete database.

Trisomy and monosomy for a whole chromosome were the first cytogenetic mechanisms leading to an abnormal phenotype to be identified. More fully, we can list the following pathogenetic mechanisms that arise from chromosomal abnormalities:

(1) A dosage effect, with a lack (deletion) or excess (duplication) of chromosomal material, whether for a whole chromosome or a part of a chromosome (Figure **1–5**)
(2) A direct damaging effect, with disruption of a gene at the breakpoint of a rearrangement
(3) An effect due to the incongruent parental origin of a chromosome or chromosomal segment (genomic imprinting)
(4) A position effect, whereby a gene in a new chromosomal environment functions inappropriately
(5) Combinations of the aforementioned





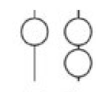


Figure 1–5
Outline of normal chromosomal dosage (two copies), versus deletion (one copy), and duplication (three copies).

We discuss these mechanisms in more detail in following chapters.

Autosomal Imbalance

Structural Imbalance

As noted earlier, imbalance may involve the gain or loss of a whole chromosome—*full aneuploidy*—or of part of a chromosome—*partial* aneuploidy. The abnormality may occur in the nonmosaic or mosaic state. Loss (i.e., monosomy) of chromosomal material generally has a more devastating effect on growth of the conceptus than does an excess of material (i.e., trisomy). Certain imbalances lead to certain abnormal phenotypes. The spectrum is listed in outline in Table 1–1 and in more detail in Table 1–2; the spectrum with respect to mental retardation is depicted in Figure **1–6**. Most full autosomal trisomies and virtually all full autosomal monosomies set development of the conceptus so awry that, sooner or later, abortion occurs—the embryo "self-destructs" and is expelled from the uterus. This issue is further explored in Chapter 23. A few full trisomies are not necessarily lethal in utero, and many partial chromosomal aneuploidies are associated with survival through to the birth of an infant.

Table 1–1. The Spectrum of Effects, in Broad Outline, Resulting from Constitutional Chromosomal Abnormality
1. Devastation of blastogenesis, with transient implantation or nonimplantation of the conceptus
2. Devastation of embryogenesis, with spontaneous abortion, usually in the first trimester
3. Major disruption of normal intrauterine morphogenesis, with stillbirth or early neonatal death
4. Major disruption of normal intrauterine morphogenesis, but with some extrauterine survival
5. Moderate distortion of normal intrauterine development, with substantial extrauterine survival and severe mental retardation
6. Mild distortion of normal intrauterine development, with substantial extrauterine survival, and considerable intellectual compromise
7. Minimal physical phenotypic effect, varying degrees of intellectual compromise; possible compromise of fertility
8. No discernible physical phenotypic effect; cognitive function within the normal range, but less than expected from the family background

Table 1–2. The Impact of Constitutional Chromosomal Abnormality on Human Mortality and Morbidity, According to Classical Cytogenetics

CONCEPTUSES OR INDIVIDUALS WITH		PROPORTION WITH CYTOGENETIC ABNORMALITY
“Occult abortion” (early embryonic death in unrecognized pregnancies)		Unknown, perhaps a quarter to a half
Miscarriage (recognized embryonic and fetal death (≥5 weeks gestation)		About 30% total. Rate varies from 50% at 8–11 weeks to about 5% in stillbirths (≥28 weeks)
Infant and childhood deaths		5%–7%
Structural congenital malformations		4%–8%
Congenital heart defects		13%
Multiple (three or more) birth defects and mental retardation		5.5%
Mental retardation	IQ < 20	? 3%–10%
(excluding fragile X)	IQ 20–49	12%–35%
	IQ 50–69	? 3%
Other neurodevelopmental disability		? 1%–3%
Criminality (defined by presence in security setting)		
Males in “ordinary” prisons		0.8%
Psychopaths, retarded criminals (male)		3.0%
Females in prison		0.4%
Male infertility (13% in those with azoöspermia)		2%
Defect in sexual differentiation (male)		<25%
Ovotesticular disorder of sex development		25%
Defect in pubertal development (female)		27%
Primary ovarian deficiency		65%
Multiple miscarriage		2%–5%

? indicates a less certain estimate.

Source: From Hook (1992).

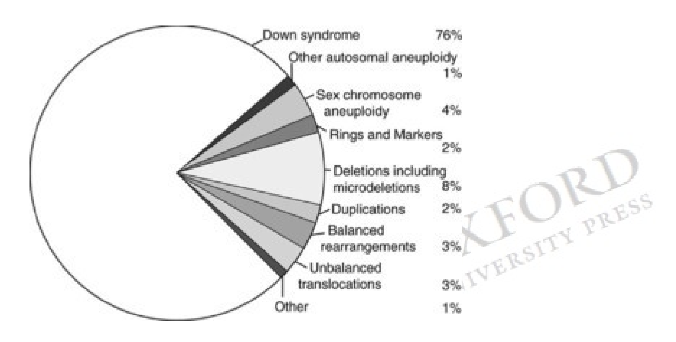


Figure 1–6
The relative proportions of different cytogenetic categories in 835 karyotypically abnormal individuals of a mentally retarded population in South Carolina studied in 1989–1994 (Phelan et al., 1996). If the exercise were to be repeated now, a lesser fraction due to Down syndrome might possibly be expected, due to prenatal diagnosis and pregnancy termination; and a new category would be needed for microduplications and microdeletions detected on microarray cytogenetics. (Courtesy M. C. Phelan; reproduced with the permission of the Greenwood Genetic Center.)

Characteristically, “survivable imbalances” produce a phenotype of widespread dysmorphogenesis, and there may be malformation of internal organs and limbs. It is often in the facial appearance (*facies*) that the most specific physical abnormality is seen. The most complex organ of all, the brain, is the most vulnerable to a less than optimal genetic constitution; and some compromise of mental and intellectual functioning, usually to the extent of an obvious deficit, is nearly invariable. While the physical phenotype in some cases of subtle deletion or duplication may be rather “bland,” compromise of neurological functioning is typical (Curry et al., 1997). Indeed, with the advent of microarray analysis, developmental delay or mental retardation[2] in the absence of dysmorphism is becoming recognized as a chromosomal phenotype (Shevell et al., 2003; Shaffer et al., 2005; Macayran et al., 2006; Hochstenbach et al., 2009). The behavioral phenotype of autism is a frequent concomitant of imbalances at both the classical and

microarray level (Rosenfeld et al., 2010a; Betancur, 2011). Thus, the central concern of most people seeking genetic counseling for a chromosomal condition is the fear of having a child who might have a physical, intellectual, or severe social handicap.

Functional Imbalance

A correct amount of chromatin does not necessarily mean the phenotype will be normal. Inappropriate inactivation, or activation, of a segment of the genome can distort the genetic message. Some segments of the genome require only monosomic expression, and the homologous segment on the other chromosome is inactivated. If this control fails, both segments can become activated, or both inactivated; and the over- or underexpression of the contained loci can cause phenotypic abnormality. The classic example of this is genomic imprinting according to parent of origin, and we discuss this concept in Chapter 22. A rather specialized example arises with the X-autosome translocation. A segment of X chromosome can fail to be inactivated; or conversely, X-inactivation can spread into an autosomal segment (Chapter 6).

Sex Chromosomal Abnormality

Sex chromosome (gonosome) imbalance has a much less deleterious effect on the phenotype than does autosomal aneuploidy. The X chromosome is one of the larger and is gene-dense; the Y is small, comprising mostly heterochromatin, and carries very few genes. In both male and female, one, and only one, completely functioning X chromosome is needed. X chromosomes in excess of one are almost always inactivated, as the normal 46,XX female exemplifies. With X chromosome excess or deficiency, a partially successful buffering mechanism exists whereby the imbalance is counteracted in an attempt to achieve the same effect as having a single active X. In such states as, for example, XXX, XXY, XXXX, XXYY, and XXXXX, excess X chromosomes are inactivated. In the 45,X state, the single X remaining is not subject to inactivation. If an abnormal X chromosome (e.g., an isochromosome, or a deleted X) is present, then, as a rule, cells containing this abnormal chromosome as the active X are selected against, perhaps due to preferential growth of those cells in which it is the normal X that is the active one. In X imbalance, the reproductive tract and brain are the organs predominantly affected. The effect may be minimal. As for Y chromosome excess, such as XYY, there is a rather limited phenotypic consequence, but again the brain may be a vulnerable organ.

The Frequency and Impact of Cytogenetic Pathology

According to the window of observation, chromosomal disorders make a greater or lesser contribution to human mortality and morbidity. Looking at prenatal existence, the earliest window has been provided by the in vitro fertilization (IVF) clinic, from the procedure of preimplantation genetic diagnosis (Chapter 26), at which single cells taken from 3-day-old embryos are subjected to genetic analysis; and an extraordinary fraction are chromosomally abnormal. After implantation (about day 5), and through the first trimester of pregnancy (to week 13), chromosomal mortality is very high, and aneuploidy is the major single cause of spontaneous abortion (Chapter 23). Perinatal and early infant death has a significant chromosomal component, of which trisomies 18 and 21 (although the latter less so in more recent times) are major elements.

As for morbidity, chromosomal defects are the basis of a substantial fraction of all intellectual deficit, and many of these retarded individuals will also have structural malformations that cause functional physical disability. Among a mentally retarded population, Down syndrome is the predominant contributor in the fraction who have a chromosome abnormality, while the increasing ability to pick up subtle deletions puts this category in second place (Fig. **1–6**). Adolescence is a period in which many sex chromosome defects come to light, when pubertal change fails to occur; and in young adulthood, chromosomal causes of infertility are recognized. Few new cytogenetic defects come to attention later in adult life, but many retarded children survive well into adulthood and some into old age, and some require lifelong care from their families or from the state. This latter group imposes a considerable emotional and financial burden. While some parents and caregivers declare the emotional return they have from looking after these individuals, for others this responsibility is a source of continuing, unresolved, if attenuated, grief.

Hook (1992) has summarized the categories of cytogenetic pathology and their impact, and we have reproduced his synopsis in Table 1–2. In Table 1–3 we set out the birth incidences of the various categories of chromosomal abnormality; these data are from a Danish study, one of a number that have examined this question in the later decades of the twentieth century, with largely similar findings in each. Overall, around 1 in 135 liveborn babies have a chromosomal abnormality, and about 40% of these are phenotypically abnormal due to the chromosome defect. If we were to look at 5-day blastocysts, the fraction with abnormality might be close to a half. If we studied a population of 70-year-olds, we could expect to see very few individuals with an unbalanced autosomal karyotype. A good approximation to a “normal” adult population—normal phenotypically and reproductively—is that reported in Ravel et al. (2006a) (Table 1–4), a study of 10,202 French sperm donors of proven fertility, bringing together data from a number of reproductive clinics comprising the “Centre d’Étude et de Conservation des Oeufs et du Sperme,” over the period 1973–2002. Donors had an upper age limit of 50 years into the 1990s, and 45 thereafter. We suggest that the somewhat lesser fractions of some of the balanced karyotypes among these men may reflect a diminished fertility due to these rearrangements, with some translocation carrier men thus ineligible to have been recruited as donors. The finer the cytogenetic focus, the greater the incidence: with the highest resolution banding and the application of molecular methodologies, a number of previously unrecognized defects would be included. It will be a task for the cytogenetic epidemiologist of this century to derive new estimates of cytogenetic abnormalities in our population.

Table 1–3. Chromosomal Rearrangements and Imbalances, Recorded in 34,910 Live Newborns in Århus, Denmark, over a Total 13-Year Period, 1969–1974 and 1980–1988

	NO. OF CASES	PER 1,000[a]	BIRTH FREQUENCY PER GROUP
Sex Chromosomes			
Klinefelter Syndrome and Variants			
47,XXY	20	1.12[b]	
47,XXY/46,XY	7	0.39	
46,XX	2	0.11	
			1 in 616 ♂
XYY			
47,XYY	18	1.01	
47,XYY/46,XY	2	0.11	
			1 in 894 ♂
XXX			

47,XXX	17	1.00	
			1 in 1,002 ♀
Turner Syndrome and Variants			
45,X	1	0.06	
45,X/46,XX and 45,X/47,XXX	3	0.18	
45,X/46,X,r(X)	1	0.06	
45,X/46,X,i(Xq)/47,X,i(Xq),i(Xq)	1	0.06	
Other Turner variant	2	0.12	
			1 in 2,130 ♀
Other			
45,X/46,XY	1	0.06	
46,XX/47,XX,del(Yq)	1	0.06	
46,XX/46,XY	1	0.06	
Total	77	2.21	1 in 453
Autosomes			
Unbalanced Forms			
Trisomy 13	2	0.06	
Trisomy 18	7	0.20	
Trisomy 21	51	1.46	
Trisomy 8	1	0.03	
Supernumerary marker, ring	25	0.72	
Deletions, duplications	6	0.17	
			1 in 379
Balanced Forms			
Robertsonian 13/14 translocation	34	0.97	
Other Robertsonian	9	0.26	
Reciprocal translocations	50	1.43	
Inversions (other than of chromosome 2)	4	0.11	
			1 in 360
Combined sex plus autosomal totals	266	7.62	1 in 131
Combined totals, excluding balanced autosomal forms	169	4.84	1 in 207

Notes: Not included in the 34,910 live newborns listing are four cases of sex chromosome induced abortion, involving the karyotypes 47,XXY, 47,XYY, 47,XXX, and 45,X/46,X,del(Xq), and 15 cases of autosomal induced abortions, involving the karyotypes +21, +13, +18, and three different derivative chromosomes. Had these pregnancies proceeded to term, the frequencies in the relevant group category would have been marginally increased.

These figures might continue to be valid into the 2000s, except that the category of deletions and duplications will substantially increase, due to the more powerful detection now offered by microarray technology.

[a] Per 1,000 male, per 1,000 female, or per 1,000 both, as appropriate. The gender-specific denominators in this study were 17,872 males and 17,038 females.

[b] An increasing incidence of XXY in recent years has been suggested, and an Australian study, including data up to 2006, arrived at a figure of 1.91 per thousand (Herlihy

and Halliday, 2008; Morris et al., 2008; Herlihy et al., 2010).

Source: From Nielsen and Wohlert (1991).

Table 1–4. Chromosomal Rearrangements and Imbalances, at a 400–500 Band Level, Recorded in a France-Wide Study of 10,202 Fertile Men (Sperm Donors) over a 30-Year Period, 1973–2002

	NO.	PER 1,000
Robertsonian 13/14 translocation	7	0.67
Reciprocal translocations	5	0.5
Autosomal inversions[a]	5	0.5
47,XYY	5	0.5
47,XYY/46,XY	1	0.1
47,XXY/46,XY[b]	2	0.2
Supernumerary marker[c]	4	0.4
Total	29	2.84

[a]Excluding three cases of the common inv(2) and one of the common inv(10)(p11.2q21.2).

[b]Excluding four cases of low level mosaicism, judged likely to have reflected cultural mitotic errors.

[c]Including two cases of der(15) marker and one of iso(Yp) mosaicism.

Source: From Ravel et al. (2006a).

The Research Application of Cytogenetic Pathology

The phenotypes that result from chromosome abnormalities can point the way to discovery of the causative genes. An early example of deletion mapping is the recognition that the gene for retinoblastoma was on chromosome 13, given the association of this cancer with the 13q– syndrome. Another cancer gene to be similarly mapped was *APC* (adenomatous polyposis coli), following the observation of polyposis in an individual with mental retardation and del(5)(q22-q23). The triple dose of chromosome 21 in Down syndrome was a signpost on the way to finding the β-amyloid precursor protein (*APP*) gene as one of the Alzheimer disease loci. A translocation with one breakpoint at 7q11.23 was found to disrupt the elastin gene in a family segregating supravalvular aortic stenosis. Further investigation of this locus in Williams syndrome proved this to be the site of deletion in this condition (Nickerson et al., 1995). We have conducted a review of chromosomal conditions in which epilepsy is a feature, with the aim of providing leads to epilepsy genes (Singh et al., 2002a). The precision of microarray analysis, coupled with access to genome databases, now allows a much finer focus in the pursuit of causative genes. For example, Ou et al. (2008b) propose that one of the genes *SIX1*, *SIX6*, or *OTX2* may be the basis of one form of branchiooto-renal syndrome, from their study of a child with a duplication of 14q22.3-q23.3.

It is a general principle that many important scientific discoveries are made serendipitously; or, as Louis Pasteur put it, “chance favors the prepared mind” (*le hasard ne favorise que les esprits préparés*). Voullaire et al. (1993) identified a small supernumerary marker chromosome (sSMC) in a child with a nonspecific picture of physical abnormality and intellectual deficit, which had no C-band positive centromere (only a constriction). Conventional wisdom has it that a chromosome cannot be stably transmitted at cell division if it has no centromere. These workers studied this sSMC and discovered that it did have a simple, but nevertheless functional centromere. This observation led the way to the delineation of the “neocentromere” (p. 307). This elemental structure could be used as the basis for designing an artificial centromere, a necessary component of a human artificial chromosome (HAC). HACs may have a potential medical role as vectors for therapeutic genes.

Historically, the chromosomal basis of some clinical syndromes has been identified following high-resolution analysis of cohorts of patients with similar phenotypes, or through the serendipitous identification of a visible chromosome abnormality. This “phenotype-first” approach led to the identification of many of the classical microdeletion syndromes. However, with the advent of microarray analysis, new syndromes are being identified based on their DNA aberration (Shaffer et al., 2007a,b). This “genotype-first” approach has led to the discovery of a number of new microdeletion syndromes, representative examples of which are reviewed in Chapter 19. In the genotype-first approach, the phenotypes may vary considerably, making it difficult to describe a distinctive clinical syndrome. A similar variability is being uncovered in some of the classical syndromes, as the wider net that can be cast due to microarray brings in atypical patients for whom targeted fluorescence in situ hybridization (FISH) would not have seemed appropriate. With the use of whole genome scans, new syndromes are being identified, unappreciated clinical variation of classic syndromes is being uncovered, and the chromosomal etiology of some known syndromes is being discovered.

Ethical and Counseling Issues

Our focus in this book is on the biology of chromosomal defects and the reproductive risks they may entail. Certain bioethical issues, coming to be more formally defined in the late twentieth century, do, however, demand attention. Counselors must hold fast to these requirements: *(1)* that they act beneficently toward their patients;[3] and *(2)* that they strive to make their services accessible to those who may need them.

Nondirective Counseling

In a Western ethos, the counselor is required to respect the autonomy of the client, and this largely translates into the principle that counseling be nondirective. Counseling may in fact never be truly nondirective, and we need to have an awareness of our own biases in order that our advice will be, as seen by those to whom we give it, valid. Rentmeester (2001) comments that, since it is “impossible for human language to convey facts purely, without any spoor of values,” and since “risk cannot be appreciated without consideration of values,” it is neither helpful nor possible to try to be value-neutral. There is a fine line between directive and detached counseling, a point nicely illustrated in Karp's (1983) deft essay “The Terrible Question” (required reading for every counselor). Ingelfinger (1980) comments, admittedly in a somewhat different context: “A physician who merely spreads an array of vendibles in front of the patient and then says, ‘Go ahead and choose, it's your life,’ is guilty of shirking his duty, if not of malpractice.” Rentmeester offers the refreshing advice that it is not necessarily unprofessional to answer a patient's question: “What would *you* do?” It is the skill of the

counselor that helps clients to reach the decision that is, for them, the right one, and for the clients to feel satisfied that they have done so. The subtleties and complexities of attempting to be nondirective in the setting of a prenatal diagnosis clinic are discussed by Anderson (1999), who analyzes responses of couples who did or who did not choose to have testing. She emphasizes the wide range of beliefs and values that people can have, as well as the likelihood for failed communication if these differences are not appreciated.

In some other societies, the perceived good of the group may carry more weight than the professed wishes of the individual. The degree to which one society can seek to influence practice in another is a matter of some controversy, well illustrated by the response in the West to the "eugenic" Chinese Maternal and Infant Health Care Law of 1994 (*Lancet* editorial, 1995). The subtleties of the issue led to keenly pointed argument (correspondence in the *American Journal of Human Genetics*, 65, 1197–1201, 1999). Knoppers (1998) comments upon the subtle boundary between the need to respect cultural, religious, and social diversity, and the imperative to adhere to tenets of generally accepted rights and ethics. More provocatively, she points to a "political and moral one-upmanship" which has colored the argument, and which may confuse deciding between what is "immoral state policy or just plain common sense."

Testing Children

To state the obvious, familial rearrangements are familial. It is very natural that parents would be concerned whether children they already have might be carriers, once an abnormality has been identified in one of them. Children, certainly, need to know their carrier status, sooner or later. It is very unfortunate (and possibly creates an exposure to legal redress) if a failure to transmit information leads to another affected child unknowingly being born elsewhere in the family. Burn et al. (1983) reported a family with a translocation having been the cause of cri du chat syndrome in two generations, the genetic information not having flowed through to the people who really needed to know it. We have had a similar experience: a family with a t(4;12) concerning which we had gone to the lengths of deriving and publishing a recurrence risk figure (Mortimer et al., 1980), and yet this information not traveling with a young man who had moved, as a child, from one country to another, and whose life has since been blighted by having had a daughter with partial 4p trisomy, and whose wife has had terminations due to unbalanced forms identified at prenatal diagnosis.

On the other hand, genetic counselors are attuned to the principle of not taking away a child's right to make, in the fullness of time, his or her own informed decision to learn about genetic risks he or she may face; thus, the principle is that the child's future autonomy is to be respected. The American Society of Human Genetics and the American College of Medical Genetics (1995) have determined that "timely medical benefit to the child should be the primary justification for genetic testing in children and adolescents," and it is true that a balanced chromosomal rearrangement will have no influence upon a person's physical health, other than, in due course, his or her reproductive health (and the issue is thus to be seen in a different light than testing for adult-onset disease). Questions are raised that testing could damage a child's self-esteem, distort the family's perceptions of the child, and have adverse effects upon the child's capacity to form future relationships (Clarke et al., 1994).

Parents' views are not without validity. Clayton (1995) comments that there is the possibility of conflict with parents, as physicians come increasingly to act as advocates for the child's interests, but notes further that "children are generally ill-served if their parents feel they have not been listened to"; she also draws the conclusion that this is a medico-ethical rather than a medico-legal issue. McConkie-Rosell et al. (1999) sought opinions from a group of 65 parents of fragile X children attending a national conference in Portland, Oregon, in 1996. They noted a "strong belief in a parental right to make the decision regarding carrier status in their children," with about half considering that they should have the right to decide when their child should be tested and informed of the result. The Genetic Interest Group in the United Kingdom gently chided the profession in commenting that "the vast majority of people are better able to understand the implications than they are often given credit for" and has enunciated the following principle: "After suitable counseling, parents have the right to make an informed choice about whether or not to have their children tested for carrier status. Ideally, children should only be tested when of an age to be involved in the decision" (Dalby, 1995). It may be that earlier concerns overstated the potential for harm: at least with respect to the mendelian cancer-predisposing syndrome familial adenomatous polyposis, children having undergone predictive testing and receiving a positive gene test result experienced no increase in anxiety, depression, or loss of self-esteem (Michie et al., 2001). Indeed, Robertson and Savulescu (2001) see potential benefit to the child, and they support the view that, as a general rule, the parents' views should prevail, and a request for predictive testing be respected. There is also the practical point that many parents will have had a prenatal karyotype from amniocentesis or chorionic villus sampling; and it may not seem entirely logical to decline to test a postnatal child.

From the foregoing, we conclude that a conservative stance, but not an immovable one, is appropriate. Debating the issue with them, many parents will see the wisdom of the declared position of the profession and be well satisfied (and possibly relieved) with the advice to leave testing until the child can decide. Equally, there will be occasions when acquiescence to a parental request may be reasonable. Either the parent's mind is set at rest, or they know of the need to raise the issue with the child at a "suitable age," which should be with the assistance of the genetic counseling clinic. The task for the counselor is to assist parents in deciding what age would be suitable for their child—"there is no universal 'right' age," as McConkie-Rosell et al. (2002) comment with respect to fragile X carrier testing—and to convey the information in such a way that concern for the future is kept in perspective, and the child's self-confidence is kept intact. And the pragmatic imperative: the wish to avoid family distress due to avoidable births of abnormal children in the next generation, as outlined earlier. Bache et al. (2007) found that 9% of carriers in Denmark, identified in childhood (or prenatally), had not been told as young adults; this observation led to a change in practice in that country, with a reminder letter being sent to the parents when their child reached the age of 18 years.

Family Studies

More widely, the parents' siblings and cousins could be carriers. Grandparental karyotypes may be useful in knowing which branch of a family to follow. The rights of individuals could, potentially, clash with the obligation that flows from belonging to a family: "no man is an island, entire unto himself" and some may see altruism as a duty. Austad (1996) proposes that the family's right to know about "sensitive genetic information" should take precedence over the individual's right not to know. He considers it "alarming to use the principle of autonomy to renounce the co-responsibility for others, in this case, relatives"; and goes on to state that "we cannot exclude ourselves from the genetic fellowship of fate into which we are born." If counselors take pains to provide clear information and to do so sensitively, such studies should usually proceed without unfortunate consequence. A suitable approach, in most families, will be to ask the person coming to the clinic to take the responsibility of bringing the matter to the attention of relatives, with appropriate support from the counselor. A letter couched in terms that it could be shown to other family members, and providing contact points for further information, is often useful. Forrest et al. (2007) reviewed many international sources and identified these criteria seen as common obligations falling to the families, and to the counselors who see them: *(1)* individuals have a moral obligation to communicate genetic information to their family members; *(2)* genetic health professionals should encourage individuals to communicate this information to their family members; and *(3)* genetic health professionals should support individuals throughout the communication process.

Predictive Gene Testing: Deliberate and Inadvertent

Counselors are very familiar with the concept of predictive genetic testing, that is to say, offering genetic testing to people who are presently well, but who are at risk for having inherited a particular genotype that may, at some stage in adult life, be the basis of the onset of disease. Its widest application is in the fields of cancer genetics and neurogenetics. With respect to translocations in the balanced state that may confer a predisposition to cancer, mention is made on p. 111, and over and above the reproductive implications of individuals being tested in such families, a cancer-associated risk will need to be assessed. As for inadvertent testing, we may mention a 30-year-old woman we have seen, presenting with premature ovarian failure and having a karyotype to check for an X chromosome mosaicism, but in whom trisomy 8 and a 14q;18q translocation were seen in 3/100 cells. She was otherwise in good health. This may well have been an "accidental" very early diagnosis of a lymphoma, and referral to a hematologist-oncologist—which was more than she had bargained for by having the test—was duly arranged. Concerning a neurogenetic focus, the delineation of the adult-onset neurodegenerative disorder associated with the fragile X premutation, and the premature ovarian failure in the female, add a layer of complexity to counseling issues with fragile X families.

With the increasing application of microarray technology, the likelihood of discovering an incidental abnormality may now need more frequently to be taken into account, when a chromosome test is ordered. Schwarzbraun et al. (2009) report their experience in testing a severely mentally retarded and mildly dysmorphic 7-year-old girl, in whom microarray revealed a de novo microdeletion (774 kb; contained 47 genes) at 17p13.1, and this deletion presumed to be the explanation for the clinical picture. One of the deleted 47 genes, however, happened to be *TP53*, and thus this deletion was considered to represent, effectively, a germline Li-Fraumeni[4] mutation. This was quite

unanticipated information for the parents to deal with, and the issue was further complicated by the child's mental incapacity. Schluth-Bolard et al. (2010) consider this question, and they write "… the local Ethical Committee at the University Hospital of Lyon, France, suggested implementation of a plan to inform patients and their parents on the possibility of discovering pathology unrelated to mental retardation, and give them a month to carefully ponder on the possible consequences before signing the consent for study." More pragmatically, they continue: "If this period of reflection would be difficult to apply in clinical practice, the possibility of incidental findings should be discussed during pre-test counseling and information should be given during post-test counseling by a trained clinician, aware of the potential psychological impact of such findings."

Netzer et al. (2009) tested a 5-year-old boy with mental retardation and identified a maternally inherited 24 kb intragenic deletion in the *PARK2* gene, mutation in which is the basis of autosomal recessive juvenile Parkinson disease, and in which a risk to the heterozygote for typical late-onset Parkinson disease may also apply. These authors discuss the pros and cons of acceding to a parental request for having themselves tested in such a situation; there is no clear answer. We have dealt with the case of a child presenting with a clinical diagnosis of Beckwith-Wiedemann syndrome, in whom microarray revealed a maternally inherited 24 kb duplication at 11q12.2 that included the *DAGLA* gene. Elsewhere, we had proposed that duplication of this gene is a plausible candidate as the basis of spinocerebellar ataxia type 20 (Knight et al., 2008); thus, the question arose as to whether we might have inadvertently done a "predictive test" for this adult-onset neurological disorder, in the mother and child. The question was further nuanced by an uncertainty relating to the role of this gene, and thus whether any further family testing would be a research versus a clinical exercise.

"Guilt" in A Carrier

Sometimes a chromosomal diagnosis may be made in an older child or even an adult, where the parents will have held for years to the notion that obstetric misadventure, or a virus, or some other blameable event was the cause of the child's condition. Some people find it upsetting to have to readjust, and to know that they may have been the source of the abnormality. They are likely to use words like *guilt*, *blame*, and *fault*. Helping these people to adjust to the new knowledge is a challenge for the counselor. They may eventually come to find the chromosomal explanation valuable and a source of some relief (as indeed some do at the outset).

Mental Retardation and Genetic Abortion

Intellectual deficiency is a condition for which many parents are unwilling to accept a significant recurrence risk—hardly remarkable, since intellectual function is such an obvious attribute of humanness. The great majority of those who chose to have prenatal diagnosis opt for pregnancy termination if a chromosomal condition implying major mental defect is identified. Some for whom abortion is not acceptable may nevertheless choose prenatal diagnosis for reassurance, or for the preparedness that certain knowledge can allow. Community views on mental handicap are changing and the late twentieth century saw something of an exodus from institutions and from special schools, as the mentally and psychologically disabled joined the "mainstream," some more successfully than others. Many syndromes, in this Internet age, have their own support groups, and these are often a source of advocacy. Counselors need to handle the tension inherent in these views and the views of parents who want to avoid having a handicapped child; and the separate conflict that parents experience when a decision is taken to terminate an otherwise wanted pregnancy. As we discussed earlier, the doctrine of nondirective counseling is a central tenet of modern practice; and it is a test of counselors' professionalism that their own views not unduly influence the advice and counsel that they give. De Crespigny et al. (1998) document the experiences and comments of a number of couples in their book *Prenatal Testing: Making Choices in Pregnancy*, intended for the lay public. Walters (1995) and Tillisch (2001) offer personal perspectives. First, Walters:

> Defending the right of women who are carrying babies with Down's syndrome to have abortions is not pleasant. Anyone who does so is likely to sound heartless, especially if they have no first-hand experience. It is even harder for me. I am the father of a Down's syndrome baby…. It is the most painful thing I will ever say but my wife, Karen and I wish she had had a test. If she had, we would have terminated the pregnancy. I must be a callous swine, mustn't I? … Her birth was a tragedy, but not so different to any tragedy that can strike out of the blue, such as a crippling accident. Just as we work to avoid other tragedies, I see nothing wrong in using Down's tests to avoid the tragedy of human handicap…. I know that I would rather not have existed at all than to be, like her, sentenced to a life of confusion, frustration, pain and possibly loneliness when Karen and I are gone. If I feel guilt, it is that I was responsible for her birth. To me that guilt is far worse than anything I would have felt had I prevented it.

Tillisch is the mother of a child with the del(1)(p36) syndrome (p. 311). Anomalies had been detected on ultrasonography during the pregnancy, but an amniocentesis returned a normal cytogenetic result. The child had a stormy neonatal course, and in due course the chromosomal defect was identified. Tillisch writes:

> I'm so thankful that the amniocentesis results were inaccurate. Since we didn't learn of Kasey's diagnosis until she was 9 months old, we were able to get to know, love, and admire Kasey as an individual, as our daughter. We didn't allow doctors to define her for us…. From a mother's perspective, Kasey's future is bright. She receives treatment and will soon go to a public school. We will allow Kasey to show us her potential, rather than labeling her "severely mentally retarded" and casting her off to be locked away from society…. My father once asked, if I could ever make Kasey "whole," would I? Without any hesitation, I answered: absolutely not. Adding the missing genes would make Kasey a different person, a stranger.

These differing, one could say polar views of parents find some parallels in the positions of those whom we could consider as the philosophers of our profession. Lejeune, in a provocative address to the American Society of Human Genetics in 1970, deplored the application of his original cytogenetic discovery to the prenatal diagnosis of Down syndrome. Epstein (2002) reflected, some three decades later, upon Lejeune's influence, and while not stepping back from the standpoint that prenatal diagnosis is a proper and valid medical procedure, he does acknowledge (as must we) that a plurality of views exists, and that the genetics community must be sensitive to, and must respect, the range of views in the community.

Brock (1995) discusses the philosophy of "wrongful handicap," addressing the question of whether *not* producing a child who would suffer has harmed that potential child; and he enunciates a principle that "individuals are morally required not to let any possible child for whose welfare they are responsible experience serious suffering or limited opportunity if they can act so that, without imposing substantial burdens or costs on themselves or others, any alternative possible child for whose welfare they would be responsible will not experience serious suffering or limited opportunity." This position could be seen as providing an ethically based framework for making a decision to terminate an abnormal pregnancy and to conceive again.

Pregnancy and The Mentally Retarded

One issue to test the caliber of the bioethicist (not to mention the counselor) is that of the rights of the intellectually handicapped to have children (Elkins et al., 1986a). What of the person with Down syndrome, or some partial trisomy compatible with fertility, in whom a question of procreation arises? Zühlke et al. (1994) give an example in describing a man with Down syndrome who developed a relationship with a mentally retarded girl living in the same house. She requested removal of an intrauterine contraceptive device, became pregnant, and the normal baby is being brought up by the maternal grandmother. On the one hand, the right of the handicapped person to experience parenthood is debated; and the American Academy of Pediatrics (1990) expressed reservation about the sterilization of intellectually handicapped women on the basis of anticipated hardship to others. On the other hand, Gillon (1987) notes that normal people have the option of being sterilized, and the mentally handicapped should have the same right. The Law Lords in Great Britain concur that sterilization may be in the best interest of the handicapped person herself (Brahams, 1987).

Many parents or guardians, not wishing to become "parental grandparents," favor sterilization. Some regard hysterectomy as having the double benefit of ensuring sterility and facilitating personal hygiene; others consider only reversible contraception to be acceptable. The High Court of Australia decided in 1992 that the parents of a handicapped child cannot themselves lawfully allow sterilization, but that a court authorization is required, and noted that this requirement "… ensures a hearing from those experienced in different ways in the care of those with intellectual disability and from those with experience of the long term social and psychological effects of sterilization" (Monahan, 1992). Ten years later, it appeared that very few unlawful sterilizations of minors were being performed in the state of Victoria (Grover et al., 2002).

When a retarded woman with a chromosomal defect is pregnant, or is pregnant by a retarded man, one or other of the couple having an unbalanced karyotype, and the

pregnancy is recognized in time, the grounds for termination are substantial. The ethical issue arises over the difficulty (or impossibility) of securing the woman's informed consent versus the expressed wishes of her guardians. Martínez et al. (1993) report from Alabama a mother with cri du chat syndrome, who was severely retarded and had no speech, pregnant by an unknown male, and "although pregnancy termination had been desired by the patient's grandmother, social and legal limitations prevented access to this procedure." Some less severely affected persons (if they are able to grasp the issue) may not regard it as undesirable to have a child like themselves; on the other hand, they may have the insight to recognize their own deficiency and not wish to pass it on. We may perhaps read this into the brief report of Bobrow et al. (1992) of a man with Down syndrome fathering a child, the mother having had first-trimester prenatal diagnosis (the baby was normal). There is the concept of imagining what a retarded person would want, were he or she intellectually competent to make a decision—a concept some would regard as paternalistic (and infringing personal autonomy) and that others see as valid and common sense. The sociology rather than the biology will exercise the counselor's mettle in this uncommonly encountered situation.

The other party is the child. Is having good parenting a right? What of a normal child born, say, to a man carrying a dup(10)(p13p14) chromosome and a mother with idiopathic mental defect? How can the interests of the child and of the parents be resolved? This is an actual case that we have seen (Voullaire et al., 2000a): it was quite poignant as this mildly retarded man, who had some insight into his own handicap, struggled to understand how best he might be a father to his 46,XX baby and expressed sadness at the abnormal behavior displayed by his older 46,XY,dup(10) child. The capable and willing grandmother stepped into the breach; but when the daughter is older, and assuming she is of normal intelligence, how will the realization of her parents' abnormality affect her? Whether a normal child in this sort of setting has a legal claim for "dissatisfied life" is an intriguing and as yet (to our knowledge) untested notion (Pelias and Shaw, 1986).

The Status of Embryos at in Vitro Fertilization

Lejeune has commented, indeed provided extensive testimony, on the ethical distinction between abortion and discarding an unwanted embryo. At a famous court case dealing with a dispute about IVF embryos in Blount County, Tennessee, in 1989, he insisted on the point that human life commences at conception, and therefore that disposing of a zygote is, in essence, no different from the induced abortion of an established pregnancy. This argument is not necessarily seen as convincing to those pragmatic couples who choose to have preimplantation diagnosis in order to avoid the predicament of having to decide upon a course of action following prenatal diagnosis of a chromosomal abnormality at chorionic villus sampling or amniocentesis. One Catholic thinker is of the opinion that "human personhood" of the embryo does not inhere until the stage at which embryonic cells have differentiated and the primitive streak has appeared (at about the end of the second week post-conception) (Ford, 1988). Prior to that time, when the "pro-embryo," as he prefers to call it, is only a *personne en devenir*, "we should resist the conceptual and linguistic temptation to attribute an unwarranted ontological unity to an actual multiplicity of developing human blastomeres." More liberally, Isaacs (2002) discusses the concept of a continuum, in which the "moral status" of the fetus increases in value through pregnancy (and indeed after birth); and some couples seem intuitively to follow this line. These issues underlie arguments about the validity or not of the term "pre-embryo" (Jones and Veeck, 2002; Tacheva and Vladimirov, 2002, et seq.)

Access to Prenatal Diagnostic Services

It would not, at present, be economically feasible or sensible to make definitive prenatal diagnosis (chorionic villus sampling or amniocentesis) available to every pregnant woman. Even among those for whom testing is, in principle, freely available, a proportion will not present, either because they are opposed to abortion, or because they have not been informed about, or have not understood, the issues involved (Halliday et al., 2001). Those who can afford it and who do not meet criteria (essentially maternal age or other particular indicators of risk) for acceptance in the public system may have the privilege of access to private testing. In the United Kingdom, the question has been raised that a wider availability, although at the expense of a lesser detection rate, would follow the implementation of a rapid diagnostic test targeting the major aneuploidies (qualitative fluorescent polymerase chain reaction [QF-PCR], FISH) (Mackie Ogilvie et al., 2009). Mass screening methodologies (Chapter 24) are to some extent bypassing the inequity inherent in the public/private dichotomy. If noninvasive early prenatal diagnosis (Chapter 25), using such methodologies as the analysis of fetal DNA or RNA from a maternal blood sample, becomes available, then potentially all pregnancies could be subject to chromosomal analysis; but this ready availability would, of itself, raise a question about the need for satisfactory counseling prior to undergoing such an "easy" procedure as a venepuncture (Schmitz et al., 2009; de Jong et al., 2010).

Legal barriers may arise in some jurisdictions. In the United States, as Miller et al. (2000) comment, "there is perhaps no more divisive subject than abortion." A possible ban on second-trimester (14–27 weeks) abortions would considerably affect couples having prenatal cytogenetic diagnosis, since many chromosomal abnormalities are discovered in the second trimester, and particularly in the period 14–18 weeks. Miller et al. calculated that a second-trimester ban would have a net annual cost of $74 million in the state of Michigan, and $2 billion in the United States, based on the estimated lifetime costs of individuals with various congenital defects (including other than chromosomal).

If prenatal testing is not made available, or if an abnormal result is reported but has not been passed on to the parents, the option of pregnancy termination is denied. Here, the legal concept of the "right not to be born" may be invoked (Weber, 2001). The issue is controversial.[5] French courts made landmark decisions in 2000 and 2001 in which substantial financial compensation was granted to parents of children with Down syndrome. Whatever the legalities, the lesson for the counselor is that testing should be offered to those for whom it may be appropriate, and that they should be diligent and careful in ensuring that prenatal testing results are safely conveyed to the right person.

Participation in Research

There is much yet to learn about clinical cytogenetics, and much of this cannot be done without patient participation (a rather obvious statement, and one that applies to medicine generally). It is, of course, well enshrined that patients who are potential recruitees should be fully informed upon the implications for themselves of a study in which they might be invited to participate, and that they have the opportunity to decline, without compromise of their own health care. Having made that point, one can see a reciprocity in providing a health care service: the patient who benefits (possibly at the expense of the state) could be seen as having a moral duty at least to consider an invitation to be involved in a bona fide research study. And having made that point, the reality is that, rather often, patients are very willing to come forward, and they gain some satisfaction in feeling that they may be making a contribution toward the greater good: the altruism gene shines brightly in many people.

It was thus disappointing to read in Giardino et al. (2009) (and see p. [link]), that a large study on de novo rearrangements detected at prenatal diagnosis could not be properly completed, in which data on a little over a quarter-million pregnancies were accumulated, from several Italian cities, and a good number (246) of de novo rearrangements identified. Here was an opportunity to build on the remarkable work of Warburton (1991). But, as these authors write, "Unfortunately, our limited information regarding the frequency and type of clinical features associated with the prenatal detection of apparently balanced rearrangements did not allow us to improve prenatal genetic counseling by updating the risk provided so far by Warburton." One perfectly valid reason may have been the logistics: " … the diagnostic laboratories, the services providing genetic counseling and follow-up and the hospitals where the births take place are not integrated, but often topographically [geographically] distant." Organizing multicenter research, and undertaking field work to gather data, is certainly challenging. However, it appeared that privacy concerns trumped any other issue: "Furthermore, request of further information in the absence of a specific consensus is forbidden by the actual [present] privacy law."

And it did not escape notice that, in the same issue of *Prenatal Diagnosis* in which this paper appeared, another paper (Ramsay et al., 2009) examined the attitudes toward research participation of parents whose child had had an abnormality shown at prenatal ultrasound. To quote these authors: " … the balance falls between the possibility of causing upset to parents, particularly those with handicapped or ill children, and the possibility of gaining new knowledge that may prove important to parents deciding whether or not to continue their pregnancy after diagnosis of a fetal abnormality." Their study in fact demonstrated "that the great majority of respondents indicated they would be happy to be contacted to provide information on their children's health and development … Research ethics committees can be reassured that the risk of causing inappropriate and unnecessary parental distress by inviting them to take part in such studies is low."

Notes:

1 Among the "al." was Marthe Gautier, who recounted, half a century following this report, her own less than fully acknowledged role in the endeavor; and Sir Peter Harper, in a commentary, and in his role as historian to the genetics community, takes an interpretative perspective upon this pioneering discovery (Gautier and Harper, 2009).

2 Words can be powerful, and choice of language can help, or hinder, a counseling consultation: facts are to be conveyed clearly but also sensitively. The reader will have noticed our use of the expression “mentally retarded” in a number of places in this chapter. Some may have flinched; others may simply have accepted this as an accurate description. “Developmental delay” is a widely used term, and it can be perfectly appropriate in a pediatric setting, although less so in dealing with an adult. In the introduction to their paper on array analysis and karyotyping, Hochstenbach et al. (2009) refer to “idiopathic developmental delay (in infants <3 years of age) and mental retardation (in older children)”; and this distinction acknowledges that prediction of intellectual capacity is more precise in older children. And yet “mental retardation” has acquired a pejorative and somewhat harsh sense over the years, and some will prefer to use such expressions as “intellectual disability” or “cognitive compromise.” As we write elsewhere, counselors will need to know to whom they speak, and what language is best to use.

3 There seems no completely satisfactory word to use here, and we variably write of patients, clients, counselees, men and women, people, and “those whom we see.”

4 Li-Fraumeni syndrome is a dominantly inherited cancer-predisposition syndrome, due to *TP53* germline mutation, with severe implications. The cancers include, in early childhood, soft-tissue sarcoma, in later childhood, osteosarcoma, and in young adulthood, breast, brain, and hematological malignancy. It is controversial whether surveillance should be offered in childhood.

5 A claim for “wrongful life” concerning cri-du-chat syndrome was brought on behalf of the child in a legal case in Australia, whose birth followed a failed vasectomy (Watson, 2002). The claim failed, the judge finding it impossible to compare, and to place values upon, impaired existence versus nonexistence.









Oxford Medicine









Chromosome Abnormalities and Genetic Counseling (4 ed.)

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Chromosome Analysis

Chapter: Chromosome Analysis
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ON CLASSICAL METHODOLOGY, chromosomes are analyzed in the cytogenetics laboratory under the light microscope at a magnification of about 1000×. The chromosomes must be stained to be visible, and a great many staining techniques have been used to demonstrate different features of the chromosome. We list some of these, in particular those with a more immediate practical application to the clinical issues we discuss in this book, or which are of historical value, when referring to the older literature.

Classical Cytogenetic Analysis

1. *Plain staining* ("solid staining"). Many histologic dyes, including Giemsa, orcein, and Leishman, stain chromosomes uniformly. Until the early 1970s these were the only stains available.
2. *Giemsa* or *G-banding*. This procedure requires a trypsin (protein digestion) step and is the main staining method in use in routine classical cytogenetics. It allows for precise identification of every chromosome and for the detection and delineation of structural abnormalities. At the 400–550 band level, rearrangements down to about 5 megabases in length can be discerned, at least in regions where the banding pattern is distinctive. Its precision is increased by manipulations designed to arrest the chromosome in its more elongated state at early metaphase or prometaphase—high-resolution banding. Alternative methods to demonstrate essentially the same morphology are quinacrine or Q-banding and reverse or R-banding. In R-banded chromosomes the pale staining regions seen in G-banding stain darkly, and vice versa.
3. *Constitutive* or *C-banding*. This technique stains constitutive heterochromatin—mainly the centromeric heterochromatin, some of the material on the short arms of the acrocentric chromosomes, and the distal part of the long arm of the Y chromosome. Constitutive heterochromatin, by definition, has no direct phenotypic effect and, in general, is devoid of active genes.
4. *Replication banding*. This technique is used primarily to identify inactive X chromatin. A nucleotide analog (BrdU) is added either as a pulse at the beginning, or toward the end of the cell cycle, to allow the cytogenetic distinction of chromatin that replicates early from that which replicates late. It produces a banding pattern similar to that of R-banding.
5. *NOR (silver) staining*. This stain, of largely historic interest now, identifies nucleolar organizing regions (NOR), which contain multiple copies of genes coding for rRNA, and are sited on the satellite stalks of the acrocentric chromosomes; these can also be demonstrated on FISH.
6. *Distamycin A/DAPI staining*. This fluorescent stain identifies the heterochromatin of chromosomes 1, 9, 15, 16, and Y. A particular use is to distinguish the inverted duplication 15 chromosome (p. 306) from other small marker chromosomes.
7. *Fluorescence in situ hybridization* (FISH) and variations thereupon. The major cytogenetic advance of the 1990s was the ability to identify specific chromosomes and parts of chromosomes by in situ hybridization with labeled probes. It is widely used to detect submicroscopic deletions and to characterize more obvious chromosome anomalies. The hybridization method may be direct or indirect. Direct attachment of a detectable molecule (e.g., a fluorophore) to the probe DNA enables its microscopic visualization immediately after its hybridization to the target DNA in the chromosome. The more sensitive indirect procedure requires special modification of the probe with a hapten detectable by affinity cytochemistry. The most popular systems are the biotin-avidin and digoxigenin systems. By using combinations of biotin-, digoxigenin-, and fluorophore-labeled probes, multiple simultaneous hybridizations can be done to locate different chromosomal regions in one preparation (multicolor FISH).
A more focused use of FISH is in the assessment of imbalances revealed by microarray analysis (see later discussion), with the probe from the genomic region targeted to the specific region identified by the array.
8. *Submicroscopic telomeric analysis*. The subtelomeric regions are, in general, gene rich, and very small rearrangements in these regions can have a profound effect. Probes have been developed for targeted subtelomeric FISH that may identify subtle rearrangements not detectable on routine banded analysis. The frequency of subtelomeric abnormalities, identified by FISH in the cytogenetics laboratory, is estimated to be about 2.5% of individuals tested (Ravnan et al., 2006). Because the subtelomere FISH assay is labor intensive and limited to only the ends of the chromosomes, this analysis has been largely replaced by microarray-based analysis.
9. *Comparative genomic hybridization (CGH)*. In CGH, differentially labeled, fluorophore-tagged DNA from the patient and a normal control (reference sample) is applied to a metaphase slide prepared from a "standard" normal person. Relative excesses and deficiencies of patient DNA bind competitively with respect to the control onto the reference chromosomes and yield different color intensities on exciting the fluorophores. This procedure has even been applied successfully to archival pathology material. It may have application in preimplantation genetic diagnosis, where it offers the advantage of testing the whole karyotype. "High-resolution" CGH refers not to a more stretched chromosome preparation, but to a further level of sophistication of the computer software that is used to analyze the images, by adjusting for the idiosyncratic patterns that each homolog may have. Small imbalances may be identifiable by this approach, ~10 Mb or greater, and the nature of uncertain rearrangements clarified (Knight & Flint, 2000; Kirchhoff et al., 2001; Ness et al., 2002). The ability to detect interstitial abnormalities offers an advantage over subtelomeric FISH (Kirchhoff et al., 2004).
Chromosomes examined by various techniques are illustrated in Figures **2–1**. Full detail is to be found in Keagle and Gersen (1999), Blancato (1999), Mark (2000), and Miller and Therman (2001), while Trask (2002) provides a historical span of the cytogeneticist's skill.

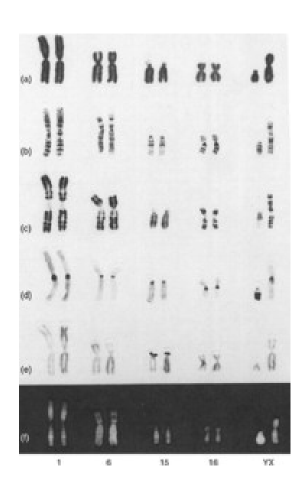


Figure 2–1
Chromosome pairs 1, 6, 15, 16, and Y and X stained by various techniques: plain stain (*a*), G-banding (*b*), replication banding (*c*), C-banding (*d*), Ag-NOR stain (*e*), and Q-banding (*f*).

Microarray Analysis

Having held sway for the better part of a half-century, classical cytogenetics is now yielding primacy of place to the power of molecular methodology, in the form of microarray analysis. Indeed in 2010, a consensus statement over the names of 32 geneticists from several countries was entitled 'Chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies' (Miller et al., 2010); and from the Professional Practice and Guidelines Committee of the American College of Medical Genetics, 'cytogenetic microarray analysis is recommended as a first-line test in an individual with multiple anomalies not specific to a well-delineated genetic syndrome, apparently nonsyndromic developmental delay/intellectual disability, and autism spectrum disorders' (Manning and Hudgins, 2010). Not that classical cytogenetics is likely to fade from view: there are two crucial reasons for its continuing use in the laboratory. First, not all array results can give a definitive construction, and FISH is often necessary to elucidate the cytogenetics. Second, the array cannot detect balanced rearrangements, and recognition of the carrier state will continue to need an old-fashioned chromosome test. And third, a rather subjective "reason" is that, by continuing to work with chromosomes, the molecular cytogeneticist will not lose the intuitive understating of what chromosomes are really like, and not see them merely as theoretical constructs from which the DNA has been extracted. The reporting of microarray results is a sophisticated exercise, and counselors need to be sophisticated readers of these reports; many laboratories now use depictions from one of the genome browsers to illustrate the precise extent of the imbalance, and noting the genes contained within this segment.

There are basically two types of microarrays available at this time: those that use a CGH approach, much like that described earlier for chromosomal CGH, and those that use single nucleotide polymorphisms (SNP) to assess the number of alleles in a sample. Although microarrays can differ in their genomic composition and substrates used for the analysis, most microarrays are comprised of thousands of spots of reference DNA sequences, applied in a precisely grided manner upon a slide (or "chip") in which the locations can be known by computer analysis.

Comparative Genomic Hybridization

The fundamental principle is essentially the same as in chromosomal CGH, noted earlier: using the array, rather than the metaphase spread, as substrate. Patient and control DNA are labeled in two different fluors, usually one that appears red and one that appears green. These labeled DNAs are applied to the microarray, and hybridization takes place. Typically, if the number of copies between the control and the patient are the same, the spot looks yellow (produced from an overlapping of equal amounts of red and green). The fluorescent intensities of each dye are measured. If the patient has an excess at a locus (due to duplication or aneuploidy), the hybridization will more reflect the dye of the patient's DNA. If the patient has a deficiency at a locus (loss due to deletion or unbalanced translocation), the hybridization will more reflect the dye of the control DNA. These fluorescent intensities are presented as a ratio of each of the dyes and plotted as shown in Figure **2–2**. Microarrays for CGH are typically constructed from bacterial artificial chromosomes (BACs) or oligonucleotides. Each spot represents a unique BAC or oligo. For example, an array with 3000 BAC spots could detect unbalanced rearrangements at a 1 Mb resolution across the entire genome (Snijders et al., 2001). The power of array-CGH over classical cytogenetics is illustrated in a study from Finland, in which ~20% of 150 patients with mental retardation, and whose G-banded karyotypes had previously been assessed as normal, showed a presumed pathogenic imbalance on microarray (Siggberg et al., 2010).

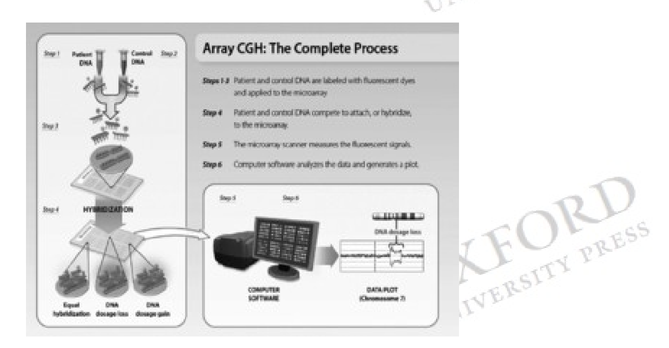


Figure 2–2
Laboratory process for microarray analysis by comparative genomic hybridization. *See also separate color insert*.

Single Nucleotide Polymorphism

Like the microarrays described earlier for CGH applications, single nucleotide polymorphism (SNP) arrays can be used to detect the number of alleles in a specimen. Heterozygosity, with two distinct alleles, can be distinguished from homozygosity, and from three alleles. Apparent homozygosity may indicate a loss of DNA, such as a deletion, while three alleles may indicate a gain of DNA copy number, such as a duplication or trisomy. SNP-based microarrays have the added advantage of detecting

uniparental disomy when the child's results are compared to the parental genotypes. Isodisomy may be revealed, in the absence of parental samples, when the entire chromosome shows homozygosity and chromosomal monosomy is an incompatible interpretation.

Balanced rearranged chromosomes, it is to be noted, cannot be detected using any of the current microarray-based technologies. The only exception, though it is not amenable to diagnostics currently, is the technique of array painting. This technology combines the use of flow sorted chromosomes to separate the two derivatives of a balanced translocation, amplifies the DNA, and applies each amplified derivative to a microarray to determine the breakpoint locations and size of the segments involved (Gribble et al., 2004).

Polymerase Chain Reaction–Based Applications

A number of technologies are available to assess DNA copy numbers. These are targeted approaches to answer a specific question: How many copies of the target are present in the patient? These techniques include quantitative fluorescent polymerase chain reaction (QF-PCR) and multiplex ligation-dependent probe amplification (MLPA). QF-PCR and MLPA use specific primers to amplify segments of DNA to determine copy number and identify deletions or aneuploidy.

Since telomeric imbalances may be relatively frequent, and often undetectable even by high-resolution cytogenetics, approaches have also been devised to assess these regions by DNA-based methodologies instead of FISH or microarrays. They have the particular advantages that all subtelomeric regions are assessed in a single test, and that several samples can be run together as a batch. One approach is that of *multiplex amplifiable probe hybridization* (MAPH), in which genomic DNA is analyzed with a set of small (140–600 bp) probes for subtelomeric sequences from every chromosome, and the degree of amplification quantified (Hollox et al., 2002). Greater or lesser amplification indicates a duplication or deletion on that particular chromosome arm.

"Next-Generation Sequencing"

DNA methodologies based on massively parallel genomic ("next-generation") sequencing have enabled remarkable advances in mutation analysis, with the entire expressed genetic complement, the "exome", tractable to interrogation. In the cytogenetic field, this approach is showing promise as a first trimester screening test for trisomy 21 that could potentially avoid invasive prenatal diagnosis, by measuring the amount of chromosome 21 DNA of fetal origin in the maternal plasma (see p. [link]).

Chromosomes, as viewed in this new century, are beginning to resemble the graph in Figure **2–3**, and the interpretation will be based upon this sort of raw data. Although cytogenetics will continue to evolve, whatever techniques come to be used, the fundamental purpose of the cytogenetic report will of course remain the same. Descriptions about the technologies used will be important addenda to reports, because they may inform the clinician about the interpretation of the chromosome analysis, and the need for further possible analysis. Reports may also include a listing of presumed significant genes in the region, a comment upon imprinting, and the likelihood of benign versus causative genomic changes. Regardless of the technology used, visualizing the karyotype by banding or FISH is, we propose, an essential part of the interpretation, and critical to enabling well-founded genetic counseling for the family (Bui et al., 2011).

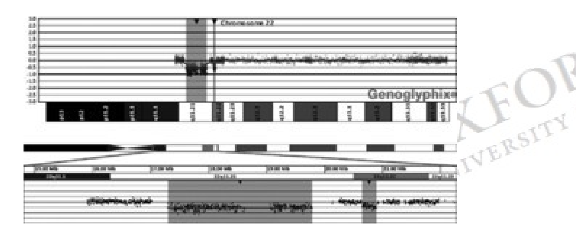




Figure 2–3
Plot of chromosome 22 in a patient with a 22q11 deletion performed using an oligonucleotide-based array with comparative genomic hybridization. The deletion is indicated by the shaded area, which reflects a deviation from the $\log_2$ ratio of 1 (equal to zero). Distal to the classic diGeorge deletion is a common copy number variant (CNV).

Genetic Counseling Considerations

In the majority of cases, the abnormalities found by molecular technologies have clear clinical relevance for the patient. However, higher resolution strategies will uncover DNA changes of unclear clinical significance. Such findings may require testing of additional family members, parents, grandparents, and sometimes siblings, to understand the relationship, if any, between the DNA alteration and the clinical phenotypes or medical problems of the patient. The possibility of findings of unclear clinical significance should be discussed when ordering the test, especially in the prenatal setting. Because these molecular-based tests have the ability to interrogate the entire genome, the pretest genetic counseling should include information about uncovering unwanted information, such as loci that can predispose to cancer loci, or to adult-onset disorders. The use of SNP arrays may uncover substantial stretches of homozygosity due to consanguineous or even incestuous relationships (Schaaf et al., 2011). These counseling caveats notwithstanding, the higher resolution potential of these new technologies will make a significant impact on our ability to make diagnoses, will increase the detection rate of chromosome abnormalities, and substantially improve our ability to provide the answers that families seek.







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Chromosome Abnormalities and Genetic Counseling (4 ed.)

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The Origins and Consequences of Chromosome Pathology

Chapter: The Origins and Consequences of Chromosome Pathology
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“WHAT WENT WRONG? And will it happen again?” These are the common questions from “chromosomal families” that bring people to the genetic clinic. We can recast these questions: “Did I, or one of us, produce an abnormal gamete? If so, why? What gamete might be produced next time? Or, if the chromosomes were normal at conception, what went wrong thereafter?” To deal intelligently with these questions, the counselor needs a broad knowledge of how gametes form, how chromosomes behave, and how the early conceptus grows. We consider the distinction between abnormality due to structural defect (full or segmental aneuploidy), the majority, and the small fraction due to functional defect (aberrant imprinting status). Most of the chromosome abnormalities in individuals that counselors see in the clinic will have arisen from errors during formation of the germ cells, and we focus particularly upon meiosis, the specialized cell division of gametogenesis. Chromosome defects can arise postzygotically, and abnormalities of mitotic cell division in the cleavage-stage embryo and in the embryo proper can produce chromosome mosaicism; we review the possible consequences of this. We refer in passing to the concept of dynamic mutation, but we leave its fuller discussion for the fragile X chapter (Chapter 15).

First, we look at *etiology*. We discuss three chromosomal settings within which genetic abnormality may arise, namely meiosis, mitosis, and genomic imprinting. Within each, we consider what types of abnormality may happen. In meiosis and mitosis, irregular segregation can produce aneuploidy for a whole chromosome, while asymmetric segregation of a structural rearrangement produces an incorrect amount of part of a chromosome (partial, or segmental aneuploidy). In genomic imprinting, the defect is qualitative, with abnormal expression of what can be a normal amount of chromosome. Sometimes there is overlap: for example, a meiotic error can subsequently lead to an abnormality of imprinting. Sometimes we cannot be sure which is the correct category: a supposed meiotic error, for example, could actually have arisen in a premeiotic mitosis. Nevertheless, this format is not too arbitrary, and it provides a useful framework within which the generality of chromosomal abnormality can be appreciated. Second, we consider *pathogenesis*: the process by which the underlying genetic defect then leads to phenotypic abnormality. Third, and with particular reference to the question of *recurrence risk* advice, we make some general comments about which categories of abnormality are likely to recur, or for which sporadic occurrence is the rule.

Meiosis

Meiosis in Chromosomally Normal Persons

The purpose of meiosis is to achieve the reduction from the diploid state of the primary gametocyte (2n = 46) to the haploid complement of the normal gamete (n = 23), and to ensure genetic variation in the gametes. The latter requirement is met by enabling the independent assortment of homologs (the physical basis of Mendel's second law),[1] and by providing a setting for recombination between homologs. While we do not dwell on recombination per se, this is, to the classical geneticist, a raison d'être of the chromosome: “from the long perspective of evolution, a chromosome is a bird of passage, a temporary association of particular alleles” (Lewin, 1994).

The mature gamete is produced after the two meiotic cell divisions: meiosis I and meiosis II (Fig. **3–1**). In meiosis I, the primary gametocyte (oöcyte or spermatocyte, also referred to as primordial germ cells) gives rise to two secondary gametocytes, each with 23 chromosomes. These chromosomes have not divided at the centromere, and they remain in the double-chromatid state. In meiosis II, the chromosomes of the secondary gametocyte separate into their component chromatids. In the male, the daughter cells produced are the four spermatids, which mature into spermatozoa. In the female, the daughter cells are the mature ovum and its polar bodies. (In fact, it is not until sperm penetration that meiosis II in the ovum is completed.) Each gamete contains a haploid set of chromosomes. The diploid complement is restored at conception with the union of two haploid gametes. The moment of conception, as the embryologist sees it, is not at sperm penetration, but only when the two pronuclei have fused to form a single nucleus (“syngamy”).



Figure 3–1
Outline of chromosomal behavior and distribution during gametogenesis. Each primary spermatocyte divides symmetrically at the sequential meioses into four spermatids. Division of the öocyte is asymmetric as it buds off the first polar body (PB1) at meiosis I, and the second polar body (PB2) at meiosis II. (The first polar body may or may not divide at meiosis II; as shown here, it has not).

Note that spermatogenesis divides the cytoplasm evenly, so that after meiosis II there are four gametes of equal size. The sperm head that penetrates the ovum comprises almost entirely nuclear material; the tail is cast off. In oögenesis, cytoplasmic division is uneven, producing a secondary oöcyte and first polar body after meiosis I, and the mature ovum and second polar body at meiosis II. The chromosomes of the first polar body may or may not undergo a second meiotic division (which would in any event be a pointless achievement). The ovum and its polar bodies each have a haploid chromosome set, but the ovum retains almost all of the cytoplasm.[2] Another major sex difference concerns the timing of gamete maturation. In the female, meiosis is partway through, in the late prophase of meiosis I, by the eighth month of intrauterine life (the actual process of recombination takes place during weeks 16–19 of fetal life). At birth, on average there are somewhat over half a million oöcytes (Bukovsky et al., 2004). Most of this pool gradually disappears, but those eggs destined to mature stay in a “frame-freeze” until they enter ovulation, some one to five decades thereafter, and meiosis recommences. Testicular stem cells, on the other hand, do not begin to enter meiosis until the onset of puberty. Thereafter, millions of mature sperm are continuously produced.

We now examine more closely the details of meiosis. During the final mitotic division in the primary gametocyte, the homologous pairs of chromosomes have (as with any mitosis) replicated their DNA to change from the single-chromatid to the double-chromatid stage. They now enter into the meiotic cell cycle (Fig. **3–2a**). As meiosis I proceeds to prophase, chromosomes conduct an “homology search” and come together and pair, with matching loci alongside each other (Fig. **3–2b**). This process—*synapsis*—continues with a more intimate pairing of the homologs, starting at the tips of the chromosomes and proceeding centrally (Barlow and Hultén, 1996), and the *synaptonemal complex* is formed. The paired chromosomes themselves are called *bivalents*.[3] Synapsis sets the stage for an exchange of matching chromosome segments; this is the process of *recombination*, or *crossing-over* (Fig. **3–2c**). Next, desynapsis occurs (the diplotene stage), with dissociation of the synaptonemal complex and the formation of chiasmata. Now, the two homologous chromosomes *disjoin* and go to opposite poles of the cell. This is the anaphase stage; the orderly movement of chromosomes during this sequence is facilitated if synapsis, recombination, and chiasmata formation have proceeded normally. Finally, the cell divides into the two daughter cells (Fig. **3–2d**). How the chromosomes are distributed—which chromosome goes to which pole—is called *segregation*. Normally, each daughter cell gets one of each of the pair of chromosomes, and this is referred to as 1-to-1 (1:1) segregation. Uniquely in the meiosis I cell division, daughter cells are produced with double-chromatid chromosomes.

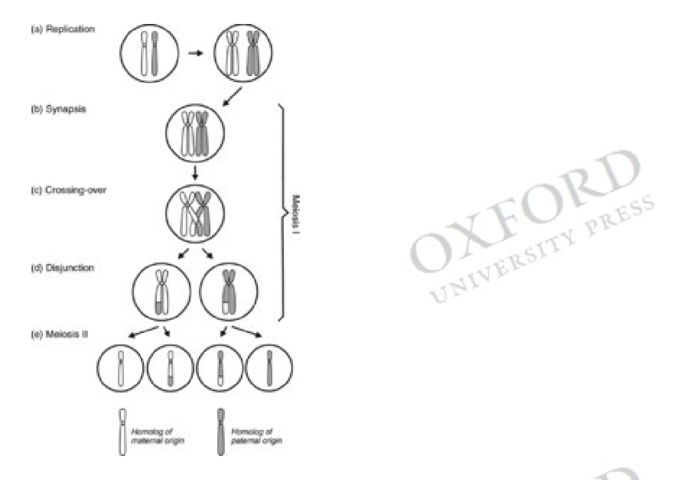




Figure 3–2
Closer detail of chromosomal behavior during meiosis I. One crossover has occurred between the long arms of one chromatid of each homolog. In öogenesis, one of the two cells at (*d*) would be the first polar body.

These cells then enter meiosis II (with the possible exception of the first polar body, as noted earlier). In this cycle, the chromosomes do not replicate, because they are already in the double-chromatid state. The chromosomes separate at the centromere, and the resulting single-chromatid chromosomes disjoin, one going to each pole, resembling a mitotic division (Fig. **3–2e**). The course of meiosis is discussed in much fuller detail in Miller and Therman (2001).

Chromosomal pathology arises when these processes of disjunction and segregation go wrong—*non*disjunction and *mal*segregation.

Meiosis in Chromosomally Abnormal Persons

Two main categories fall under this heading. The *first*, and most important, is the phenotypically normal person heterozygous for a balanced structural rearrangement (translocation, inversion, and insertion being the major forms). *Second*, there is the rare instance of persons who are themselves chromosomally unbalanced with either a full or a partial aneuploidy, and thus mostly phenotypically abnormal, and who present with questions of their reproductive potential. We will deal in detail with each situation in separate chapters, but we will consider the broad principles here.

Balanced Carriers

In heterozygotes for some balanced rearrangements involving only small segments, the chromosomes may "ignore" the nonhomologous material they contain, and pair (this is "heterosynapsis") and segregate much as would happen at a normal meiosis. In other balanced rearrangements, the inherent tendency to pairing dictates that homologous segments of rearranged chromosomes will align, as well as they are able to achieve this ("homosynapsis"). This may require the chromosome to be something of a contortionist, forming complex configurations such as multivalents and reversed loops. According to either scenario, the stage is set for the possibility of unbalanced segregation. The gametes produced—and therefore the conceptuses that arise—are frequently unbalanced. In this context, a segmental aneuploidy is usually involved, that is, a part of a chromosome is present in the trisomic or monosomic state; or, rather frequently, a combination of trisomy for one segment and monosomy for another. Partial trisomy and partial monosomy are also referred to as *duplication* and *deletion*, respectively.

In some rearrangements, recombination presents a further hazard. Inversions and insertions may produce a new *recombinant* (rec) chromosome that has a different genetic composition from that of the original rearrangement. A conceptus forming from a gamete containing it would inevitably be genetically unbalanced.

Aneuploid Individuals

In the individual who has a full aneuploidy, and in whom gametogenesis is able to proceed, in theory a trivalent may form, or a bivalent and an "independent" univalent. Either could lead, effectively, to a 2:1 segregation. This appears actually to be the case in trisomy 21; whereas in gonosomal[4] states (XXX, XXY, and XYY) the "third" chromosome is, as it were, disposed of, and the great majority of gametes are normal. In the person with a partial aneuploidy due to an unbalanced rearranged chromosome, whether 46,(abn) or 47,+(abn), the abnormal chromosome may have an even (or near-even) chance to be transmitted in the gamete; but the opportunity to observe such outcomes rather infrequently arises.

Mitosis and Mosaicism

The purpose of a mitotic cell division is faithfully to pass on an intact and complete copy of the parental cellular genome to the progeny cells. The mitotic cycle consists of the following sequence: gap-1 period (G1) → synthesis period (S) → gap-2 period (G2) → mitosis (cell division). The G1→S→G2 components together comprise the interphase period of the cell cycle. During the S period, the chromosomes replicate their DNA, thus converting from the single-chromatid to the double-chromatid state. Genetically active segments of chromosomes replicate earlier during the S period, while inactive segments, which include almost the entire inactivated X chromosome in the female, are late replicating. The cell division period is further subdivided into prometaphase → metaphase → anaphase → telophase. The chromosomes condense to enter prometaphase, and condensation continues into metaphase. Metaphase chromosomes align on the equatorial plate, and the spindle apparatus becomes attached to the centromere of each chromosome, consisting of its two kinetochores. Pulled at the kinetochores (centromeres), the chromatids of each chromosome then separate (disjoin) and are drawn in opposite directions (anaphase) and arrive at the opposite poles of the cell (telophase). Then the chromosomes decondense, the nuclear membrane reconstitutes, the cytoplasm constricts and divides, and two daughter cells now exist.

Mosaicism due to Mitotic Error

A mitotic error can cause phenotypic abnormality by generating an abnormal cell line at some point during embryogenesis. If we focus on the end result, the feature distinguishing mitotic from meiotic errors is that the former typically produce a mosaic conceptus, while meiotic errors produce a nonmosaic abnormality. We define constitutional chromosomal mosaicism as the coexistence, within the one conceptus, of two or more distinct cell lines which are genetically identical except for the chromosomal difference between them, these cell lines having been established by the time that embryonic development is complete (the point at which the embryo becomes a fetus). Thus, the different cell lines are fixed in the individual and are a part of his or her chromosomal constitution. The earlier in embryogenesis that a mitotic error occurs, the greater the likelihood for a substantial fraction of the soma to be aneuploid, leading to increasing departure from normality of the phenotype. It is probable that many mitotically arising abnormalities lead to cell death, leaving no trace.

Considering the enormous numbers of mitoses that proceed successfully, it is clear that the ordering of chromosomal disjunction during cell division must be a marvelously robust mechanism. A complex system of interacting components underlies the mechanism, including the cohesin multiprotein complex, among which are the similar RAD21 and REC8 proteins having crucial roles in mitosis and meiosis, respectively, and the synaptonemal complex proteins (Bardhan, 2010). Rare instances of marked mitotic instability indicate the existence of errors in the system, some at least of which may be genetically determined (Mikkelsen, 1966). For example, Miller et al. (1990) karyotyped a child because of major physical and neurodevelopmental defects, and he had cells trisomic and monosomic for almost every chromosome; only about a quarter were 46,XY. As a possible milder manifestation of this phenomenon, Fitzgerald et al. (1986) described a mother, herself physically normal, who had had three trisomic 21 conceptions. Her own tissues (blood, skin) were mostly 46,XX, but some cells had a variety of aneuploidies (47,+21, 47,+18, 47,XXX), indicating a proneness to chromosome malsegregation apparently operating both in meiosis and mitosis. A very early example of familial mosaicism, back in the days of solid-stain cytogenetics, concerns a family in which a mother and son were mosaic for a Robertsonian translocation, and the son had two mosaic children, a girl with Down syndrome who had four separate cell lines, and a boy with 45,X/46,XY mosaicism (Zellweger and Abbo, 1965). The more recently delineated mosaic variegated aneuploidy syndrome (p. 346) is now the classic example of a presumed "mitotic checkpoint mutation."

> The quite common finding of loss of an X or a Y chromosome in an occasional cell in an older female or male population (and more notably in centenarians) may reflect "normal" age-related anaphase lag (Guttenbach et al., 1995; Bukvic et al., 2001; Russell et al., 2007). Aviv and Aviv (1998) refer to "age-dependent hidden mosaicism" and propose a role for the progressive shortening of telomeres in leading to somatic aneuploidies in older persons. Possibly, this chromosomal change might be an agent, rather than just a passive consequence, in the ageing process; a possibility the consideration of which is well beyond the remit of this book.

A chromosome test on any normal person—a routine analysis from a sample of peripheral blood—would probably get a normal result (46,N). We would conclude from an analysis of a dozen or so cells from one specialized tissue that the rest of the soma is also 46,N. In most of the person's tissue, this will be truly the case. But the body comprises a vast number of cells—a trillion (10^{12}) or so—which required a vast number of mitoses for their generation. The dozen cells checked in the laboratory are only a billionth of a percent of all the person's cells, and we routinely (and, for practical purposes, not unreasonably) regard this minute fraction as a valid representative of the remaining 99.999999999%. Notwithstanding, we can surely suppose that one or more errors will have happened, during one or some of the many mitoses, and these will have produced a chromosomally abnormal cell line and the person is really a chromosomal mosaic. It seems plausible to imagine that unrecognized islands of mosaicism, involving a tiny number of cells—only a few thousand or a few dozen, perhaps—could well be a fairly frequent state. In fact, the cornea of the eye, and the brain, may show mosaic aneuploidy as an apparently normal phenomenon (Pettenati et al., 1997; Westra et al., 2008). Almost certainly, somewhere in their soma, everyone is a mosaic.

Detecting Mosaicism

The great majority of cytogenetic studies are done on blood samples. Blood is a specialized tissue, and it may not necessarily reflect the karyotype elsewhere in the body. Skin fibroblasts are a more "basic" tissue, and skin biopsy has long been performed in the pursuit of a diagnosis of mosaicism. A particular case is that of the Pallister-Killian syndrome, due to 12p isochromosome (p. 305), which cannot be diagnosed on blood, and thus skin biopsy is a necessary procedure. Chorionic villi and amniocytes are the tissues assessed at prenatal diagnosis, and "confined placental mosaicism" is a well-recognized category. Other somatic tissues amenable to study, and thus allowing recognition of mosaicism, are the buccal mucosal cell and the urinary epithelial cell (Reddy and Mak, 2001; Stefanou et al., 2006). A somewhat different question is mosaicism in the preimplantation embryo (see the following section).

Mosaicism from the First Divisions of the Zygote

The first few mitotic divisions from the one-cell zygote are particularly vulnerable to error, and this brief period of development needs to be considered separately. Insight into this vulnerability has come from experience in the in vitro fertilization (IVF) laboratory, with the application of preimplantation diagnosis. It may be that the early cleavage pre-embryo has to rely on an inadequate supply of maternal cell-cycle control factors, conveyed in the egg, before being able to bring about its own autonomous production. Surprisingly large fractions of pre-embryos, on fluorescence in situ hybridization (FISH) analysis, are chromosomally mosaic. In one series, for example, in which 216 apparently normal IVF zygotes were followed through to the 2–4 cell and 5–8 cell stages, almost half were mosaic (Bielanska et al., 2002b). Going from the 2–4 cell to the 5–8 cell stage, the fraction rose from 15% to 50%. Often, the mosaicism was "chaotic," that is, different cells had different aneuploidies. Pre-embryos that failed to advance had much higher levels of mosaicism than those whose development appeared to proceed smoothly, as naturally might have been expected. Munné et al. (2002b) studied over a thousand IVF embryos and deduced that a substantial fraction reflected mitotic nondisjunction, these embryos typically consisting of cells with normal, trisomic, and monosomic chromosomal constitutions. Interestingly, this category of embryo mosaicism was associated with increasing maternal age, possibly reflecting the decay of stored factors in the oöcyte as just mentioned. One must bear in mind that all these observations are made in the unnatural setting of in vitro development, and that the picture may be less abnormal in vivo. Further, FISH methodology may be less accurate than initially supposed, and mosaicism is observed much less frequently if SNP-microarray is the test procedure (Treff et al., 2010a). These matters are dealt with in some detail in Chapter 26.

Insight into the timing of the abnormality can also be gained from inference in the study of mosaic individuals. Jacobs et al. (1997), in a study of Turner syndrome, observed that patients with Xq isochromosome mosaicism hardly ever have a 46,XX cell line: most are 45,X/46,X,i(Xq). This is what would be expected if the error happened at the very first mitosis of the initially 46,XX zygote. If it happened at the next two or three divisions, a 46,XX cell line would also have been present, 45,X/46,XX/46,X,i(Xq). If three cell lines are detected, an origin in a later mitosis can be assumed. For example, Stefanou et al. (2006) describe an abnormal infant with trisomy 20 mosaicism on blood, but with a monosomic 20 cell line identified in urinary epithelial cells. The first division of the zygote may be especially, but certainly not uniquely, prone to error.

Chimerism

Chimerism,[5] which is to be distinguished from mosaicism, is the coexistence of more than one cell line in an individual, due to the union of two originally separate ("sibling") conceptions. It could be imagined that twin blastocysts happen to make contact and then fuse (IVF may facilitate this happening), and this may be the more typical scenario. Alternatively, but likely very rarely, there might have been two sperm fertilizing an ovum and a polar body. A 46,XX + 46,XX chimera would most probably present as a normal female, whereas 46,XX + 46,XY could manifest an abnormality of sexual differentiation. An extraordinary example of chimerism is recorded in Wiley et al. (2002) of a malformed stillborn with 47,XY,+21 plus 47,XX,+12.

The most usual form is "confined" chimerism, in which only one tissue—that is, blood—possesses the two cell lines. This is due to twin-to-twin (or feto-fetal) transfusion, when dizygous twins have intimately opposed placentae, allowing vascular connections ("anastomoses") to form between them, with marrow colonization by the other twin's hematogenous cells. Sudik et al. (2001), for example, describe a woman typing XY in 99% of peripheral lymphocytes on fluorescence in situ hybridization (FISH) analysis, but karyotyping 46,XX on three other tissues, including ovarian; she had had a twin brother, who had died as a neonate.[6]

Twinning

Dizygous twinning is more frequent in mothers in their late thirties, and so it is not remarkable that occasionally twins are born, one with normal chromosomes, and the other with a maternal-age-related aneuploidy. Monozygous twinning could happen in an abnormal conception just as in a normal one, and the occasional instance of twins concordant for an abnormal karyotype is to be expected (Schlessel et al., 1990). Rather more remarkable is the case of monozygous twins discordant for karyotype—clearly the adjective "identical" is inappropriate here! Rogers et al. (1982) studied monochorionic twin brothers, one 46,XY and the other 47,XY,+21 with Down syndrome, in whom marker analysis supported a diagnosis of monozygosity. The skin fibroblast karyotypes were nonmosaic, but both infants showed blood mosaicism, presumably from twin-twin transfusion in utero. In this type of twinning, the assumption is that either an initially 47,XY,+21 conceptus underwent splitting, with loss of a chromosome 21 then occurring in one of the newly created embryos; or, vice versa, a mitotic nondisjunction occurred in one monozygous embryo from an initially normal conception. Similarly, Nieuwint et al. (1999) describe two sets of monozygous twins, one of each pair being chromosomally normal and the other abnormal, monosomy X in one case and trisomy 21 in the other. Lewi et al. (2006) were able to assemble six such cases from one center, with cases of trisomy 21, trisomy 13, and monosomy X. A 47,XXY conception that led to monozygous twins of different gender, one 46,XX and the other 46,XX/46,XY, is reported in Zech et al. (2008). As for a structural rearrangement, Zeng et al. (2003) describe monozygous twins, one mosaic for a 1q duplication due to 46,X,der(Y)t(Y;1)(q12;q12), and the other twin normal; their analyses were consistent with a postzygotic error. Similarly, monozygous twin brothers, one 46,XY and the other with Xq isochromosome Klinefelter syndrome, 47,X,i(Xq),Y, are reported in Stemkens et al. (2007).

Perhaps the most extraordinary circumstance of discordance in monozygous twins concerns the acardiac (that, is, lacking a heart) fetus. Trisomy 2 is one of the aneuploidies observed (Blaicher et al., 2000). An initially normal conceptus might generate a trisomy 2 cell line that then separates and produces the co-twin; or, an initially trisomic conceptus gives rise to a "corrected" lineage. It is only the presence of the normal twin that allows the acardiac co-twin to survive, with placental vascular connections providing blood circulation from normal to abnormal twin. We have seen such a case due to trisomy 3, with the affected acephalic, acardiac fetus of barely recognizable human form.

Somatic Recombination in Homologs.

Genetic exchange can take place, as a normal event, during a mitotic cycle, either involving the pair of homologous chromosomes, or the sister chromatids of one chromosome. The cytogenetic demonstration of sister chromatid exchange (SCE) is rather dramatic (Fig. 21–2). Should the SCE be unequal, tandem duplication and deletion lines may be generated. If the deletion line is lost, a normal/duplication mosaicism results (Rauen et al., 2001). According to the somatic extent of the abnormal cell line, the phenotype may or may not be affected; and according to its involvement in the gonad, a reproductive risk may or may not apply.

Nondisjunction

Nondisjunction in Meiosis

Classical Description

Nondisjunction is remarkably frequent, and in consequence many human conceptions, perhaps about a quarter, are trisomic or monosomic. Nondisjunction is defined as the failure of homologous chromosomes to segregate symmetrically at cell division. The classical description of the mechanism of meiotic nondisjunction is as follows. In a chromosomally normal person, if the pair of homologs comprising a bivalent at meiosis I fail to separate (fail to disjoin[7]), one daughter cell will have two of the chromosomes and the other will have none. This is 2:0 segregation (Figs. **3–3a** and **3–4**, upper). In other words, one cell is disomic for that homolog, and the other is nullisomic. Nondisjunction may occur in meiosis II, meiosis I having proceeded normally. In meiosis II, it is the chromatids that fail to separate (Fig. **3–3b**). Following these nondisjunctional errors, the conceptus, at fertilization, ends up trisomic or monosomic, assuming the other gamete to be normal (Fig. **3–5a,b**). Trisomy or monosomy in the offspring of normal parents is called primary trisomy or primary monosomy.

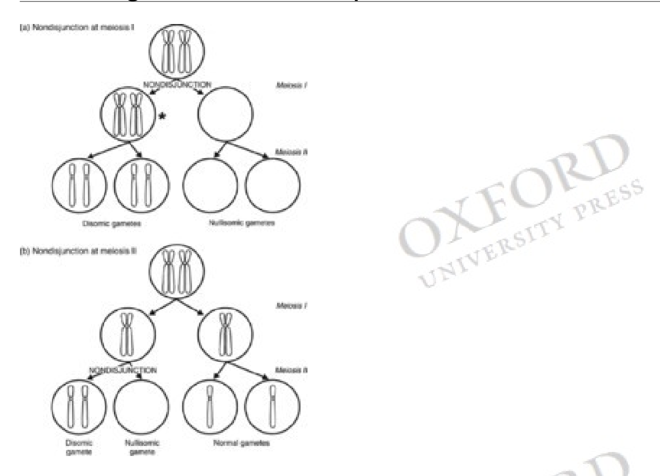




Figure 3–3
The classical view of the mechanics of nondisjunction. The asterisked gamete reflects the complement of the oöcyte in Figure **3–4** (*upper*). In öogenesis, one of the two cells following meiosis I would be the first polar body, which might or might not proceed to meiosis II.

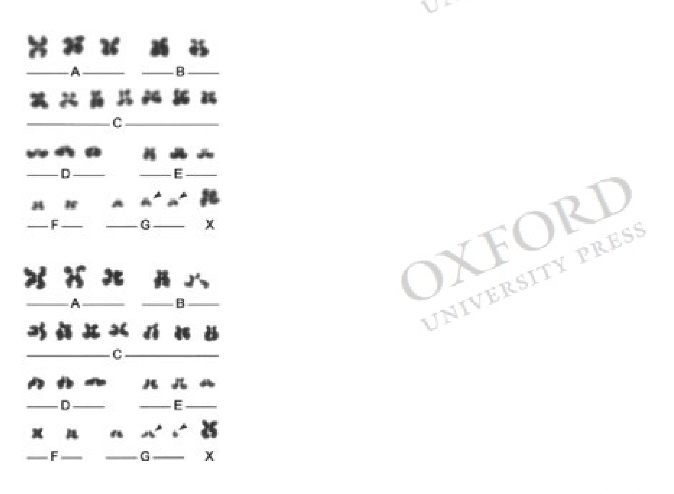




Figure 3–4
Oöcytes at metaphase of meiosis II, showing nondisjunction of a G-group chromosome having occurred at the preceding first meiotic division. *Upper*, oöcyte with classical nondisjunctional disomy, showing an additional G-group double-chromatid chromosome. Possibly the arrowed pair are chromosome 21s, and the karyotype 24,X,+21. *Lower*, oöcyte with "predivisional" disomy, showing an additional G-group single chromatid. The arrowed pair may be chromosome 21s, and the karyotype 24,X,+21cht. (From Kamiguchi et al., 1993, courtesy Y. Kamiguchi.)

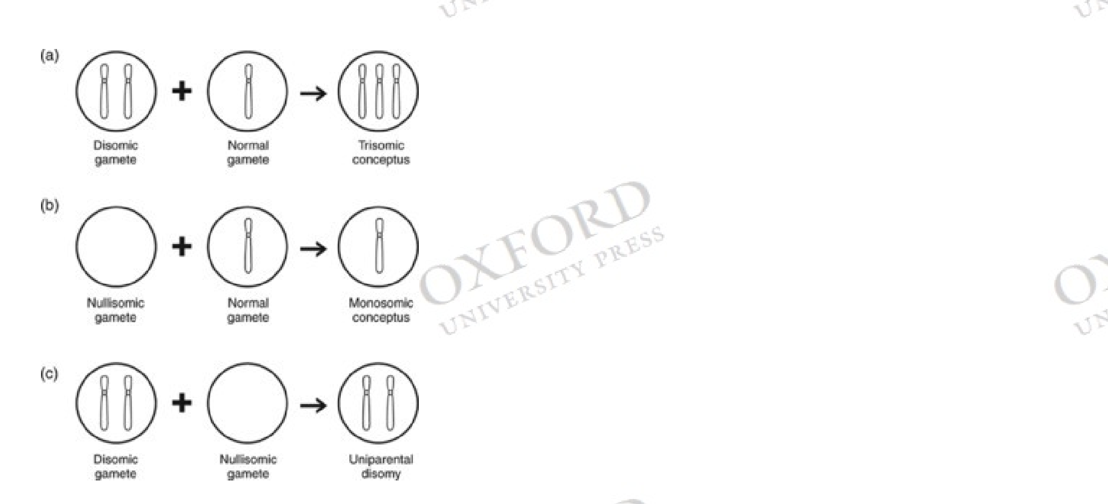


Figure 3–5
Aneuploid gametes producing an aneuploid conceptus (*a* and *b*), and aneuploid gametes producing uniparental disomy (*c*).

Most nondisjunction, at least in öogenesis, is assumed to occur at the first meiotic division. This is the conclusion reached with the most extensively studied chromosomes, nos. 8, 13, 15, 16, 18, and 21 (Nicolaidis and Petersen, 1998). Indeed, in the case of trisomy 16, every case is proposed to be due to a maternal meiosis I error. With respect to the X chromosome, about 90% of nondisjunctions leading to the 47,XXX state are of maternal origin. At least half of the maternal X nondisjunctions that cause 47,XXX and 47,XXY arise from meiosis I errors, and about a third in meiosis II (Thomas et al., 2001). Meiosis II is the site of some autosomal nondisjunctions, and in fact most trisomy 18 may result from this stage. Autosomal nondisjunction in spermatogenesis results in far fewer abnormal conceptions than from öogenesis, with the 22 autosomes mostly having about an equal likelihood to undergo nondisjunction, although some (chromosomes 16, 21, 22) appear to be more vulnerable (Shi and Martin, 2000b). Only in trisomy 2 among the autosomes is there a substantial paternal contribution, with close to half reflecting a meiotic error in spermatogenesis (Hassold, 1998; Robinson et al., 1999).

Given the frequency with which nondisjunction happens, it is not at all surprising that instances of double trisomy are known. The reader with a sense of history will want to review the 48,XXY,+21 case described in Ford et al. (1959); and a very few other cases, proceeding through to live birth, have followed, the most common combination being trisomy 21 along with an additional sex chromosome (Li et al., 2004; Tennakoon et al., 2008). The observation is more frequently made, and double autosomal combinations seen, in the analysis of products of conception from spontaneous abortions (Micale et al., 2010). Through the IVF laboratory, a very much larger number have come to light, and often with several coexisting aneuploidies; for the most part, these embryos would not even proceed through to the stage of implantation.

While autosomal aneuploidy overwhelmingly has its origin in oögenesis (and meiosis I at that), male gametogenesis has a major part to play in the generation of sex chromosome abnormalities. Obviously enough, the 47,XYY state, when it is due to a meiotic error, must be due to paternal nondisjunction (at meiosis II, logically), with the production of a 24,YY sperm. Some may represent a postzygotic error (Robinson and Jacobs, 1999). As much as half of 47,XXY Klinefelter syndrome is due to nondisjunction having occurred in spermatogenesis, giving a 24,XY sperm. X-Y nondisjunction is predisposed following an absence of recombination in the primary pseudoautosomal regions (PARs) of the X and Y at meiosis I. And monosomy X is mostly due to absence of a paternally contributed sex chromosome.

Sequential nondisjunctions at both meiotic divisions could lead to tetrasomy, and this is the basis of some X chromosomal polysomy (Hassold et al., 1990b; Deng et al., 1991). *Simultaneous nondisjunctions* of two chromosome pairs can lead to double aneuploidy, as noted earlier. *Simultaneous parental nondisjunctions*, both gametes being disomic, is rare, but not unknown, and is another route to double aneuploidy, and for example Robinson et al. (2001) describe 48,+14[pat],+21[mat] in a spontaneous abortion. If one gamete is disomic and the other nullisomic, for the same chromosome, one parent has contributed both members of the homologous pair, and the other none (Fig. **3–5c**). This is uniparental disomy due to "gametic complementation," an event of extreme rarity. *Simultaneous errors* of nondisjunction and other rearrangement would typically be quite coincidental, such as a child having both XXY Klinefelter syndrome (maternal nondisjunction), and del(15)(q11.2q13) Prader-Willi syndrome (paternal deletion) (Nowaczyk et al., 2004). *Complete nondisjunction* is an expression that could be applied in the case of triploidy, when this is due to the retention of the polar body within the ovum (Martin et al., 1991).

The Chromatid "Predivision" Hypothesis of Angell

An alternative mechanism for nondisjunction is based on the premise that precocious separation of chromatids ("predivision") during meiosis I is the crucial factor. This is Angell's (1997) hypothesis, and it is based upon direct oöcyte observations. Three sequential events comprise the gist of this theory (Fig. **3–6**). First, the (double-chromatid) homologs fail to pair during meiosis I[8]; or, if they do pair, they separate again before meiosis I is complete. In other words, instead of the two (double-chromatid) chromosomes existing as a conjoined bivalent, they exist as two separate univalents. Second, these univalents are prone to "predivide"—that is, the separation of the two chromatids that should normally happen at meiosis II instead takes place while they are still in the first meiotic cycle. This could happen to both univalents or just the one; and these would then exist as single-chromatid chromosomes. Third, at anaphase of meiosis I, these double- or single-chromatid chromosomes segregate to the oöcyte and polar body independently. The oöcyte in Figure **3–4** (lower) may be an example of asymmetric segregation due to this process, having received a double-chromatid and a single-chromatid chromosome. Sandalinas et al. (2002) provide some corroborative support for Angell's hypothesis in their findings on direct analysis of fresh oöcytes, with both predivision and nondisjunction more frequent in the gametes of women over age 35 years than under 35, and more often observed in the smaller chromosomes. Similarly, Pellestor et al. (2005) and Treff et al. (2009a) show, using oöcytes obtained during IVF procedures, that predivision is a more frequent cause of aneuploidy than is classical nondisjunction, and furthermore is related to increasing maternal age. The phenomenon may not be confined to the female: Uroz et al. (2008) observed premature chromatid separation in sperm of an infertile man.

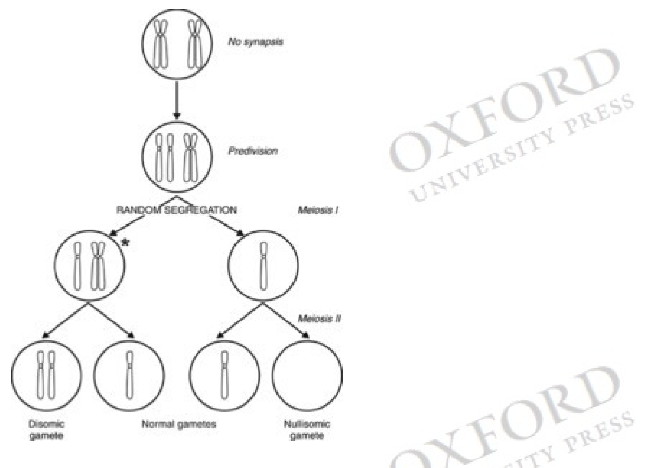




Figure 3–6
Nondisjunction following "predivision" of one homolog into its component chromatids in meiosis I (Angell's hypothesis). The asterisked gamete reflects the complement of the oöcyte in Figure **3–4** (*lower*). In öogenesis, one of the two cells following meiosis I would be the first polar body, which might or might not proceed to meiosis II.

Certain terminologies and nomenclature may be mentioned here. A gamete with an extra chromosome is *hyperhaploid*, with a karyotype written, say, 24,X,+21. A gamete missing a chromosome is *hypohaploid*. (e.g., 22,Y,–21). If, at meiosis I, the extra chromosome is present only as a single chromatid (e.g., the asterisked oöcyte in Fig. **3–6**), the abbreviation *cht* is used: thus, 24,X,+21cht. In addition, the ISCN provides nomenclature for meiotic cells, and an extra 21 at meiosis I, present as a univalent, would be denoted as MI,24,+I(21) (ISCN 2009).

Causes of Nondisjunction

The great majority of aneuploidy due to nondisjunction arises in oögenesis. A particular vulnerability of maternal meiosis may lie in the degradation, over time, of factors that underpin the adhesion of the homologous chromatids of the bivalent. This failure of snug apposition, then, leads the chromosomes to adopt unstable positions when meiosis resumes. The particular unstable position will depend, where there is just one chiasma, upon whether its site is toward the middle or toward one end of the chromosome. This can then allow the pairs of chromatids, only loosely attached to each other at this single chiasma, to act as independent univalents at the first meiotic division (Wolstenholme and Angell, 2000; Pellestor et al., 2002; Yuan et al., 2002). One apparently rare mendelian basis is the *SYCP3* gene, coding for one of the synaptonemal complex proteins, mutation in which affects meiosis both in the female, to produce aneuploid oöcytes, and in the male, to cause spermatogenic arrest (Bolor et al., 2009). Another theory has it that the motor proteins associated with the centromere may be a point of vulnerability. Chromosomes move along the spindle to their appropriate destinations by the active intervention of these motor proteins, and if they are not working properly, chromosomes may end up being not where they ought to be (Hodges et al., 2002). Quality checking, which is stringently applied in the male, is poorly effective in the female, and so the maturing of an aneuploid oöcyte is not prevented; as Hunt and Hassold (2002) suggest, Nature seems to have erred in putting less protective investment into the more scarce gamete.

While these meiosis-control factors may be the proximate cause of failed disjunction, what background attributes might lead to a loss in its integrity? Of course, advanced childbearing age is an obvious answer. A very telling insight comes from the work of Battaglia et al. (1996). These investigators sampled oöcytes at meiosis II metaphase from

younger (20–25 years) and older (40–45) volunteers who were having normal menstrual cycles. They did not look at individual chromosomes but rather at the disposition of the spindle and the metaphase chromosomes as a whole. They made the most striking findings according to the ages of the women: a symmetrical and neatly arrayed complex was seen in the younger women, while in the older women the spindle was askew and the chromosomes a-jumble, as shown in Color Figure **3–7** (see separate color insert). It is not difficult to accept that this structural disorganization would undermine the ability of the chromosomes of the oöcyte then to undergo regular segregation. And in a paper that could have been subtitled "A Tribute to the Polar Body," Fragouli et al. (2011) show how revealing can be an analysis of the chromosomes of this discarded remnant. Exploiting the fact that the polar body's karyotype is, in a sense, the opposite of that of the oöcyte, and that the first and second polar bodies reflect segregations at meiosis I and II, respectively (Fig. **3–1**), they could show that maternal age has a very strong association both with nondisjunction and with predivision of chromatids, and that meiosis II becomes, with age, the more vulnerable division.



Figure 3–7
Meiosis II oöcytes from younger and older women, illustrating what may be the physical basis of the maternal age effect. The microtubules of the spindle stain green, and the chromosomes stain orange. The tracing identifies these components, and the smooth or wavy lines suggest, respectively, an intact or a degenerating spindle apparatus (the ages of the women indicated). The chromosomes are well organized at the metaphase plate at the equator of the cells in the younger women (the 22-year-old's oöcyte, on the upper left, is viewed on a tilt). In contrast, the 40-year-old's oöcyte shows the chromosomes in disarray. The 42-year-old woman's oöcyte has one chromosome, at the top, dislocated from the metaphase plate, and the disposition of the other chromosomes at the equator is not as regular as in the younger women. (The color photographs are from D. E. Battaglia et al., (1996), Influence of maternal age on meiotic spindle assembly in oöcytes from naturally cycling women, *Human Reproduction* 11:2217–2222. Courtesy D. E. Battaglia; reproduced with the permission of Oxford University Press and Human Reproduction.) *See also separate color insert*.

Accumulation of mitochondrial DNA mutations might be a further factor causing compromise of the functioning of the support structures underpinning the meiotic mechanisms (Schon et al., 2000), and Seifer et al. (2002) demonstrated an increasing frequency with age of a particular mtDNA deletion, which is otherwise known to be age related, in the cells that surround the oöcyte within the follicle (the granulosa cells). Thus, support may be drawn for the view that a gradual deterioration in the integrity of the meiotic apparatus with increasing age of the mother is the major factor in predisposing to meiotic nondisjunction in female gametogenesis. Individual variation may mean that some women are more prone to these effects than others (Nikolaou and Templeton, 2004).

Not that the young are immune. Fragouli et al. (2006a), in a paper dedicated to the memory of the 18-year-old patient whom they had studied, analyzed oöcytes harvested ahead of her chemotherapy for a marrow malignancy which, had she lived, might have enabled fertility. Of 11 oöcytes and 7 first polar bodies able to be analyzed, one egg had a single-chromatid X and could have gone on to a monosomy X conception, while another egg was inferred (via its polar body) to have an additional X and 21 chromatid, and the conception could have been 48,XXX,+21. The introductory sentence of this paper is worth quoting: "Humans as a species are not as fertile as other mammals"; and, as already noted, it is at meiosis in the oöcyte that much of this susceptibility resides.

Nondisjunction in Mitosis

Normal Zygote

Mitotic (somatic, postzygotic) nondisjunction is the major mechanism in the causation of mosaicism. Nondisjunction can occur in an initially normal (46,N) zygote, with the generation of mosaicism for a trisomic and a concomitant monosomic line, as well as the normal line (Fig. **3–8a**). In autosomal nondisjunction, growth of the monosomic cell line is severely disadvantaged, and it will very probably die out in early embryogenesis, leaving just the normal and the trisomic cell lines comprising the individual.[9] Mosaic Down syndrome, with the karyotype 46,N/47,+21, is the classic example. In one particular autosomal aneuploidy, trisomy 8 mosaicism, somatic nondisjunction accounts for the great majority of cases (Karadima et al., 1998).



Figure 3–8
Generation of mosaicism. (*a*) Postzygotic nondisjunction in an initially normal conceptus. In this example, one cell line (monosomic 21) is subsequently lost, with the final

karyotype 46,N/47,+21. (*b*) Postzygotic nondisjunction in an initially normal 46,XX conceptus, resulting in 45,X/46,XX/47,XXX mosaicism. (*c*) Postzygotic anaphase lag in an initially abnormal 47,+21 conceptus; this leads to a “corrected,” or “rescued,” normal cell line.

Actually, about 5% of standard apparently nonmosaic 47,+21 is also due to a mitotic defect (Antonarakis et al., 1993), with the “third” chromosome 21 equally likely to be maternal or paternal. In 3% of apparently nonmosaic 47,XXY and 9% of 47,XXX, the error was postzygotic, presumably prior to the formation of the inner cell mass (MacDonald et al., 1994). As noted earlier, the nature of the mosaicism can indicate the likely time of its generation. An aberrant mitosis involving the X chromosome, in an initially 46,XX zygote, may generate X and XXX cell lines, both of which would be survivable. If this happens at the first mitosis, X/XXX mosaicism would result. If at any later mitosis, a normal cell line would exist, and the mosaic state would be X/XX/XXX (Fig. **3–8b**). The same can happen in a 46,XY zygote, with an X/XYY, or an X/XY/XYY mosaicism resulting (the gender in the embryo being determined according to the sex chromosome composition of gonadal tissue). More than one mitotic error can happen, separate in time and place; for example, DeBrasi et al. (1995) identified concomitant 45,X and 47,XX,+8 (and 46,X,+8) in a woman with clinical features of both trisomy 8 and Turner syndrome, in whom the molecular study supported the hypothesis of an originally 46,XX conception.

> Two sequential abnormal events can lead to the intriguing situation of nonidentical monozygous twins of opposite sex. The mitotic loss of a Y chromosome in a 46,XY conceptus could produce X/XY mosaicism; and then a splitting of the conceptus could produce monozygous twins in which the distribution of the X and XY lineages might differ. Costa et al. (1998) studied two sets of X/XY mosaic twins, each set comprising a girl and a boy. In one set, the girl was diagnosed clinically as Turner syndrome and had a 45,X[23%]/ 46,XY[77%] karyotype on blood analysis, while her male co-twin was 45,X[16%]/ 46,XY[84%]. In the other set, the girl had mixed gonadal dysgenesis, and her karyotype was 45,X[24%]/46,XY[76%]. Her brother's karyotype, on blood, was 45,X/46,XY with the same ratios as his sister; but on fibroblast culture he was nonmosaic 46,XY. He had absence of one testis. Fetal blood mixing, due to vascular connections in the shared placenta, may have contributed to his 45,X cell line.

A most extraordinary suggestion, and one which would overturn much of the received wisdom about the generation of trisomy, comes from Hultén[10] et al. (2008, 2010), who studied ovarian tissue from apparently normal female fetuses, and observed, in eight out of eight cases, and with a total of 12,634 cells analyzed, a very low-level trisomy 21 mosaicism: 0.5%, with a range of 0.2%–0.9%. In contrast, no such mosaicism existed in the testes of male fetuses. They speculate that, in an initially 46,XX embryo, precursor gametic cells may be vulnerable to mitotic error; and they reason from this observation that “most women may be trisomy 21 ovarian mosaics.” This they propose as the basis for the predominance of a maternal origin of the extra chromosome in Down syndrome and other aneuploidies. The age effect may be due to a different maturity of the trisomic oöcyte. This will be an interesting debate to follow.

Aneuploid Zygote

Nondisjunction can occur in a postzygotic mitosis in a conceptus that is initially trisomic for an autosome (say, 47,+21). Thus, one copy of the homolog in question is lost. The same result may be due to the mechanism of anaphase lag. (In this latter case, the chromosome fails to connect to the spindle apparatus, or is tardily drawn to its pole, and fails to be included in the reforming nuclear membrane. On its own in the cytoplasm, it will form a micronucleus and soon be lost.) This converts the trisomy in this cell to 46,N. Its descendant cells are 46,N, and the karyotype of the conceptus is, say, 46,N/47,+21 (Fig. **3–8c**). Most mosaic trisomy/disomy 13, 18, 21, and X arises in this way, for example, 47,XXY → 46,XY/47,XXY (Robinson et al., 1995).

A conceptus with what might be called “interchange tertiary trisomy”—that is, a 47-chromosome count, with the two translocation chromosomes and an additional copy of one of the derivative chromosomes—might generate a cell line with the balanced state, if one of the derivatives is lost postzygotically. Thus, a zygote with, for example, a 47,t(1;2),+der(1) karyotype might acquire a cell line with 46,t(1;2). If this cell line included blood-forming tissue, but if much of the soma otherwise consisted of cells with the unbalanced state, a phenotypically abnormal child could have, on blood sampling, a balanced translocation karyotype. Such a case is presented in Dufke et al. (2001); and speculatively, this scenario might be a rare contributor to the apparent slight excess of abnormal children among the balanced carrier offspring of translocation carrier parents (p. 109).

Postzygotic “Correction” of Aneuploidy and Uniparental Disomy.

If the conversion of trisomy to disomy—sometimes referred to as “*correction*” or “*rescue*”—occurs prior to the formation of the pre-embryo, and if the 46,N line then gives rise to the pre-embryo, the embryo will be nonmosaic 46,N. According to which one of the three chromosomes was lost, normal biparental disomy in the embryo could be restored, or uniparental disomy (UPD) could result (Fig. **3–9**). This is a much more common mechanism of UPD than the scenario of coincidental nondisjunctions in the genesis of both gametes as depicted in Figure **3–5c**. It is at prenatal diagnosis, typically, that the fact of this rescue mechanism comes to be discovered, with trisomy seen at chorionic villus sampling (CVS), and disomy at a subsequent amniocentesis (Sirchia et al., 1998). Chromosome 15 is of particular concern, and Purvis-Smith et al. (1992) and Cassidy et al. (1992) provide historic illustrations in pregnancies showing 47,+15 at CVS, with conversion to 46,N at amniocentesis; the infants had upd(15) mat, which produces the phenotype of Prader-Willi syndrome. Walczak et al. (2000) showed the same thing retrospectively, in demonstrating trisomy 15 by FISH on archived placental tissue.

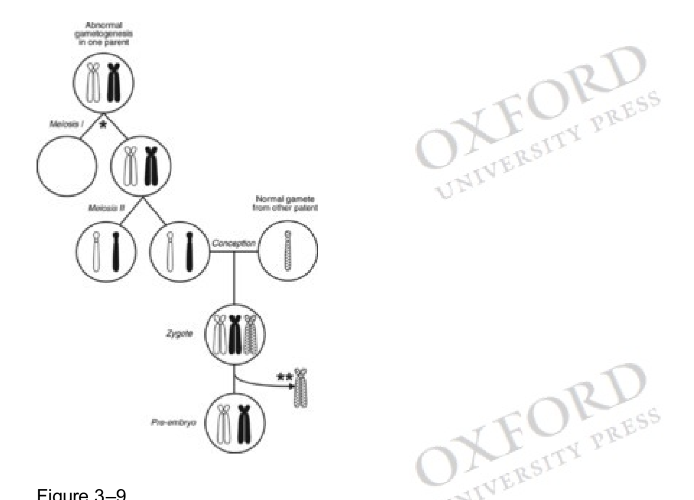
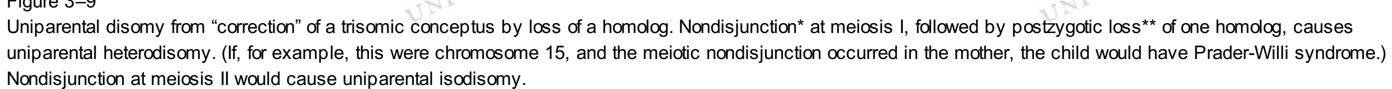


Figure 3–9



Uniparental disomy from “correction” of a trisomic conceptus by loss of a homolog. Nondisjunction* at meiosis I, followed by postzygotic loss** of one homolog, causes uniparental heterodisomy. (If, for example, this were chromosome 15, and the meiotic nondisjunction occurred in the mother, the child would have Prader-Willi syndrome.) Nondisjunction at meiosis II would cause uniparental isodisomy.

An inference of “rescue” may be made in the case of UPD discovered because of isozygosity for a recessive gene, and an example of this is deafness due to the connexin-26 gene. Yan et al. (2007) report a child presenting with deafness due to homozygosity for the 35delG mutation, for which his father, but not his mother, was a carrier. As it

transpired, the child had upd13(pat), with isodisomic and heterodisomic segments of chromosome 13, the segment in 13q12.1, which contains the connexin-26 locus, being one of the regions of isodisomy. Quite possibly, this had been a trisomic 13 conception but rescued due to discarding one of the chromosomes, which happened to be the maternal chromosome 13. Had it not been for the coincidence of the father's heterozygosity for the 35delG mutation, the rescue would have been entirely successful.

Postzygotic correction can also happen in the other direction, as it were: to convert a monosomic zygote into a disomic one. It is rarely recognized. Quan et al. (1997) report a girl, 46,XX, with Duchenne muscular dystrophy due to a homozygous deletion of exon 50 of the *dystrophin* gene. She had homozygosity of the X chromosome for all of the tested marker loci: apparently, a complete maternal uniparental isodisomy X. Even a meiosis II nondisjunction would probably have had some heterozygosity, due to recombination at meiosis I; and so Quan et al. propose a mitotic mechanism. A 45,XO conception, from a 22,O sperm + 23,X egg at syngamy, underwent duplication, or possibly nondisjunction, of the single X chromosome. Unfortunately, this X chromosome carried a de novo Duchenne mutation. It remains an open question how many monosomy X conceptions, the X chromosome being normal, are successfully corrected to produce normal 46,XX,upd females (Schinzel et al., 1993).

Structural Rearrangement

The following classical structural rearrangements may be listed: translocations, insertions, inversions, isochromosomes, duplications, deletions, and complex rearrangements. Smaller rearrangements, which may be referred to as “genomic rearrangements,” are of a size discernible on high-resolution banding (3–5 Mb), or may be of submicroscopic size (typically measured in kilobases) (Gu et al., 2008). All arose de novo at one point: whether with the index case in whom the abnormality was discovered, or in a parent or more distant ancestor, with a balanced (or, infrequently, unbalanced) form transmitted thereafter in the family. Jacobs (1981) has derived the following mutation rates for the generation of de novo classical rearrangements: 1.6×10^{-4} per gamete for the balanced reciprocal translocation, and 2.9×10^{-4} per gamete for unbalanced rearrangements (a mutation rate that is magnitudes greater than those typically seen in autosomal dominant conditions).

The illegitimate breakage and reunion that produces these rearrangements is typically due to the apposition of chromosomal segments containing DNA sequences with a high degree of homology—“paralogous sequences,” also known as “duplicons.” Palindromic AT-rich sequences comprise the basis of “hot-spots” at 11q23, 17q11, and 22q11, leading to recurrent translocations t(11;22) and t(17;22) arising with breakpoints at these sites (Kurahashi et al., 2010). Most breakpoints are in nontranscribed DNA, and thus, for the most part, contribute no untoward effect upon the phenotype. If the breakpoint in one chromosome were to disrupt a gene, phenotypic defect is the likely consequence; the Developmental Genome Anatomy Project (Higgins et al., 2008) aims to collect such cases, in order to understand the precise nature of the molecular perturbation due to the breakpoint, and the process by which the phenotypic defect arises. We discuss possible mechanisms of formation in more detail in the appropriate chapters.

Setting in Which Rearrangement Occurs

Mutations causing chromosomal rearrangement can occur during both meiosis and mitosis, and in the gonad of either sex. But (in contrast to the maternal risk for whole-chromosome aneuploidy) it is the male who is the susceptible parent, at least with respect to unique (nonrecurrent) rearrangements (Shaffer and Lupski, 2000; Kurahashi et al., 2010; Thomas et al., 2010). Balanced nonrecurrent de novo rearrangements show a paternal age effect, and likely arise at a premeiotic mitosis, whereas meiosis is the site of generation of unbalanced nonrecurrent de novo rearrangements, with no age effect discerned. Only in recurrent rearrangements, such as the Robertsonian translocation, is a maternal bias seen. A different view is proposed by Vanneste et al. (2009), who suggest that chromosomal breakage-fusion-bridge cycles in the cleavage stage (day 1–3) embryo may be a frequent mechanism for several types of rearrangement, including mosaics.

Balanced and Unbalanced Rearrangements

Structural rearrangements can be *balanced*, with the correct amount of genetic material in a cell, or *unbalanced*, with a deletion and/or duplication of genetic material. Arguing somewhat circularly, in the phenotypically *normal* person it is inferred that, although such an individual's genetic material is in a different chromosomal arrangement, it is present in the correct (balanced) amount and functioning properly. It is irrelevant to the person's health, other than his or her reproductive health. It may be helpful in explaining this to think of the person's genome as a recipe book—a series of instructions for everything that is genetically determined. If an error occurs in the pagination (a translocation), and, say, pages 17 to 24 are inserted between pages 36 and 37, the recipes are all still there; they are still perfectly capable of being read. If a sequence of pages is inserted upside down (an inversion), one need only turn the book around to read them.

At the cytogenetic level, one can only use the term “apparently balanced.” A not infrequent clinical and laboratory puzzle is the instance of a phenotypically *abnormal* individual who has an apparently balanced rearrangement. Might it be, at a submicroscopic level, that genetic imbalance really exists? Even the highest-resolution banding does not reveal a genetic imbalance at the level of a few kilobases of DNA; but now with microarray methodology, light is being cast on some of these cases, with a substantial fraction revealed as being, in fact, unbalanced (Schluth-Bolard et al., 2009; Vandeweyer and Kooy, 2009), or “molecularly balanced” but disrupting a gene that could plausibly be pathogenic (Cacciagli et al., 2010). If the (normal) parent has the apparently identical chromosome, the problem becomes more complex. These matters are dealt with in more detail in Chapter 5.

A rearrangement that is balanced at the genomic level may yet lead to a phenotypic consequence due to “position effect.” Brown et al. (2010) review examples and describe their own case of a family with a segregating paracentric inversion, inv(7)(q21.3q35). The proximal breakpoint at 7q21.3, and which was associated with a 5 kb deletion, led to loss of an enhancer of the genes *DLX5* and *DLX6*, and also caused these genes to become functionally separated from other presumed regulatory elements. As a result, the activities of *DLX5* and *DLX6* were compromised; and since these genes influence development of the inner ear and aspects of craniofacial and limb formation, deafness and facial and limb malformation resulted. This may be considered an “epigenetic” effect, and we now go on to discuss this concept.

Epigenetics and Genomic Imprinting

A formal definition of an epigenetic effect includes these points: the DNA sequence of a particular gene remains unaltered, but the ability of this gene to be expressed is altered. The expression “genomic imprinting” is applied in the setting of epigenetic effects that are applied during germline transmission. Some parts of some chromosomes are subject to genomic imprinting as a *normal* occurrence, and this imprinting is parent specific: that is, genes in the chromosomal segment are expressed, or not expressed, according to whether the chromosome had been transmitted in the sperm or in the ovum (“parent-of-origin effect”). An imprinted segment takes up an “epigenetic mark,” and the gene or genes in this segment are not expressed, leaving it to the corresponding locus or loci on the homologous chromosome from the other parent to be the only source of expression. Imprinting can therefore lead to a differential activation status of the two alleles of the locus or loci concerned: one of the pair is functional, and the other is “silent.” When the phenomenon was first appreciated in humans, it was naturally suspected that many forms of congenital abnormality might be due to aberrant imprinting. As it has transpired, however, the practical application of genomic imprinting appears to be confined to a rather small number of cytogenetic conditions. Nevertheless, the theoretical interest is considerable.

Most of the autosomal genome is not subject to imprinting, and it is functionally disomic. That is, with each locus having a pair of alleles, each of the pair is functionally active, contributing more or less equally to the genetic output from that locus.[11] This is *biallelic gene expression*. A minority of the genome is subject to imprinting and requires only one of the pair of alleles to be active, while the other one becomes inactivated; in other words, the locus is functionally monosomic, with a genetic output from only one allele. This is *monoallelic expression*. If the allele of maternal origin is inactivated, only the allele of paternal origin is functionally active; and vice versa. Following conception, the imprint remains through cycles of postconceptional somatic mitoses: the chromosome “remembers” the sex of the parent who contributed it (put differently, it retains its epigenetic mark). The imprinting pattern may be specific to a certain tissue, or to a certain developmental stage (Ideraabdullah et al., 2008). Thus, in some tissues a gene may express monoallelically, while in other tissues biallelic expression is retained; or a gene may express monoallelically in a specific tissue at one stage in embryogenesis, and biallelically thereafter. X chromosome inactivation is a special case.

Parent-of-origin imprinting is a normal mechanism of gene regulation. It is mediated through a process taking place during gametogenesis, of which the physical basis includes methylation of cytosine bases within the gene(s), or in controlling sequences upstream of it. This process is reversible, and in the "life" of an autosomal allele or chromosomal segment, as it passes from individual to individual down the generations and across the centuries, imprinting—the epigenetic mark—will be acquired, maintained, lost ("erased"), reacquired ("reset"), and lost again according to the sexes of the individual through whom it is transmitted. Throughout, it retains the same DNA sequence.

Mechanisms Whereby Functional Genetic Abnormality Can Arise

In the context of imprinting, we may consider three categories of functional genetic defect. These are as follows: uniparental disomy with overexpression and/or nonexpression of genes in certain chromosomal segments; deletion with nonexpression; and relaxation of imprinting with overexpression. *(1) Uniparental disomy* will lead to either biallelic expression, or no expression, at the locus or loci within the imprintable segment. *(2)* If a *deletion* removes a chromosomal segment that would otherwise have been "silenced," all that is lost is a nonfunctioning genetic segment, and there is no untoward consequence. On the other hand, if the deletion removes the segment on the active chromosome, the corresponding part of the other homolog is inactive, and so neither chromosome will be genetically functioning in this segment; in a sense, the silent allele is unmasked. *(3) Relaxation of imprinting* allows a segment that should be nonexpressed to lose its imprint. The locus or loci contained therein will be operating biallelically, which will be, in this case, at double normal capacity. These mechanisms are dealt with in detail in Chapter 22.

Another category of epigenetic effect is that imposed by compromise of controller elements, such that the client gene, which is of itself normal, is inappropriately nonfunctioning; the "position effect" due to a translocation separating the controller and the client has been mentioned earlier.

Trinucleotide-Repeat Transmission

Every counselor will be familiar with the concept of the trinucleotide expansion disorders. The first to have been recognized as such, in 1991, was Kennedy spinobulbar muscular atrophy, followed shortly by the fragile X syndrome. With the fragile X syndrome, the number of trinucleotide repeats can change as the allele is transmitted from parent to child. Having reached a critical number, the gene's function is compromised, and phenotypic abnormality declares itself. The rare folate-sensitive fragile sites are most likely due to expansions of naturally occurring highly polymorphic CCG sequences (Sutherland, 2003).

Consequences of Genetic Abnormality

Structural Imbalance

Chromosome imbalances are harmful because of the fundamental reason that some (not all) genes are dosage sensitive. In *duplications*, there is 150% of the normal amount of this chromosomal segment; and in the *deletion*, 50% of the normal amount. The imbalance involves a whole chromosome (*full aneuploidy*), or a part of a chromosome (*partial aneuploidy*, *segmental aneusomy*). An incorrect amount of dosage-sensitive genetic material in every cell of the conceptus distorts its development to a greater or lesser extent. Large losses or gains almost invariably set early development so awry that abortion occurs. Lesser imbalances may be compatible with continued intrauterine survival, but with the eventual production of a phenotypically abnormal child. Very minor imbalances may cause defects that are not readily detectable in early infancy; and some chromosomal "defects" may be without phenotypic effect. However, as a first principle, anything but 100% of the normal amount of (at least autosomal) genetic material produces a less than 100% normal phenotype. Mental defect is the almost universal consequence of autosomal imbalance, and vice versa, much mental defect is due to a chromosome abnormality.

> It is generally too simplistic to think of deletions and duplications leading to opposite qualities of phenotype (Neri and Romana Di Raimo, 2010). But in some instances the concept of "type and countertype," originally proposed by Lejeune (1966), may be invoked. Deletion of 7p15 may cause the cranial bones to fuse prematurely (craniosynostosis), due to abnormal behavior of osteoblasts at their periphery, whereas duplication leads to underdevelopment of the skull, with a large and confluent fontanelle (Stankiewicz et al., 2001c). Deletion of 15q26.1-qter (which removes the growth factor locus, *IGFR1*) is associated with intrauterine growth retardation, whereas dup(15)(q26.1-qter) may cause a syndrome of postnatal overgrowth (Nagai et al., 2002; Faivre et al., 2002).

Quantitative Assessment

How do we determine what is a large or a small degree of genetic imbalance? First, we can take a quantitative approach—how much material is involved. The finer detail, as measured in megabases of DNA, is known for each chromosome band (Table 3–1). Now, in the second decade of this century, as microarray methodology becomes a first-line chromosomal investigation (Miller et al., 2010), the counselor will become familiar with deletion/duplication reports couched in precise terms of start and finish basepair numbers. With respect to "classical" degrees of imbalance (several megabases), for practical purposes the blunter measurement of haploid autosomal length (HAL) is useful (see Appendix B). The largest chromosome, no. 1, comprises 8.4% of the HAL, whereas chromosome 21, the smallest, is 1.9%. As a very general rule, if the imbalance consists of less than 1% of HAL, the conceptus is often viable in utero, and live birth frequently results. If the excess is greater than 2%, in utero lethality, with spontaneous abortion, is likely. Imbalance involving autosomal deficiency (partial monosomy) is generally much less survivable than is duplication (partial trisomy).

Table 3–1. Actual Lengths of DNA Accommodated in Each Band, Measured in Bases, Using Chromosome 19 as a (Helpfully Small) Example

BAND	BASES FROM PTER TO LOWER MARGIN OF THIS BAND	SIZE OF THIS BAND
p13.3	8,362,000 bp	8,362,000 bp
p13.2	12,656,000	4,294,000
p13.13	14,916,000	2,260,000
p13.12	17,628,000	2,712,000
p13.11	22,600,000	4,972,000
p12	27,100,000	4,500,000
p11	30,000,000	2,900,000
cen		
q11	32,500,000	2,500,000
q12	36,900,000	4,400,000
q13.11	40,211,000	3,311,000
q13.12	43,221,000	3,010,000
q13.13	46,833,000	3,612,000
q13.2	49,541,000	2,709,000
q13.31	51,046,000	1,505,000
q13.32	54,357,000	3,311,000
q13.33	58,872,000	4,515,000
q13.41	60,979,000	2,107,000
q13.42	63,688,000	2,709,000
q13.43	67,000,000	3,312,000

Notes: Cytogenetic resolution is at very high level (850-band). The p terminus is in band p13.3, and the q terminus in band q13.43. The whole chromosome is 67 Mb long, 30 Mb in the short arm and 47 Mb in the long arm. The average band size is approximately 3.5 Mb. Data from Internet site of the Weizmann Institute of Science.

Qualitative Assessment

Quantitative assessment of the amount of chromatin deleted or duplicated, based on classical cytogenetics, is rather crude. In general, dark G-bands are low in gene content, and light G-bands are gene rich; the telomeric regions have the highest gene density. More precisely, Saccone et al. (1999) have determined, on the basis of probing for regions rich in GC sequences, those parts of the different chromosomes that carry a greater quantum of genes (Fig. **3–10**). Chromosomes, or chromosomal segments, of greater or lesser gene density are likely to have, in the unbalanced state, a lesser or greater survivability, respectively.

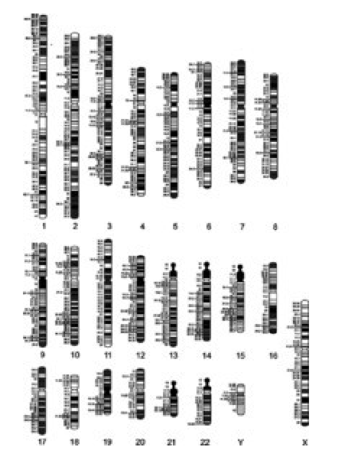






Figure 3–10

Gene-rich regions of the human karyotype. The bands in red hybridize to genomic DNA abundant in CpG islands. Note the absence or near-absence of gene-rich regions in the three “viable” autosomes, nos. 13, 18, and 21 (and cf. the reference on p. 278 to the mere 225 genes on chromosome 21). Chromosome 19, on the other hand, is well colored in, which accords with knowledge of the rarity of viable chromosome 19 imbalance. (From Figure 2 in S. Saccone et al., 1999, Identification of the gene-richest bands in human prometaphase chromosomes, *Chromosome Research* 7:379–386. Courtesy G. Bernardi; reproduced with the permission of Kluwer Academic Publishers.) *See also separate color insert*.

Assessment is made, however, not on this theory, but upon the empiric observations of phenotypes. Some segments (e.g., 9p, all of 21) appear to have a substantial pre- and postnatal survivability in the trisomic state, whereas a lesser number of segments (e.g., distal 4p) are sometimes viable when monosomic. Chromosome 13 provides the most impressive examples of viability for a large autosomal imbalance. Trisomy for the whole of chromosome 13—fully 3.7% of the HAL—frequently goes through to live birth, and in the 13q–deletion syndrome, monosomy occurs for up to 2.5% of HAL. This presumably reflects a low gene density on this chromosome (Fig. **3–10**), and a relative paucity of genes that are sensitive to dosage imbalance. The same likely applies to chromosomes 18 and 21. Occasionally, imbalance is so “small” that the effect on the child's physical phenotype is only very minor, and intellectual function can remain within the normal range, albeit toward the lower end of that range. There are some segments which, when duplicated or deleted, appear to cause no abnormality at all. For example, trisomy for the segment 9p12p21.3 (comprising as much as ~0.6% of HAL) was found in a physically and mentally normal man, who had “learned a technical profession” (Stumm et al., 2002). The concept of heritable “euchromatic deletions and duplications without phenotypic effect” is discussed in Chapter 16.

Other segments impart a serious trisomic/monosomic effect even for a tiny amount of chromatin. Deletion of the single band 17p13.3, for example, causes the severe phenotype of the Miller-Dieker syndrome. The least number of loci that can be removed in a deletion is one; in Rubinstein-Taybi syndrome, for example, the loss of a single copy of the gene *CREB* within 16p13.3 suffices to produce the phenotype.

Now, in the microarray era, a qualitative assessment may include, for smaller size segments (measured in kilobases), an illustration to accompany the cytogenetic report from one of the genome browser Web sites that shows the genes within that segment. Sometimes, a particular gene may present itself as a plausible, or perhaps the known basis of the associated phenotype; rather often, however, the list of genes will include several whose role, if any, in determining or contributing to the observed clinical picture may be quite obscure.

Karyotype-Phenotype Correlations for Deletions and Duplications

Differing lengths of deleted or duplicated segments enable a dissection of the specific segmental contributions to components of an abnormal phenotype. These various observations of karyotype-phenotype correlations permit judgment concerning whether a particular duplication or deficiency may be of minor, major, or lethal effect. A “malformation map” can be produced from documenting the association of certain congenital defects with particular segmental aneusomies (Brewer et al., 1998, 1999; Fig. **3–11**). One of the most commonly seen defects is a heart malformation. The complex twisting and folding of the cardiac tube is, apparently, particularly vulnerable to an incorrect dosage of certain genes. These genes may reside in those chromosomal regions associated with heart defects, and van Karnebeek and Hennekam (1999) have documented these associations. A “susceptible cardiological karyotype” does not, however, necessarily determine that a heart defect will happen, as attested, for example, by the observation of discordant monozygous twins with a dup(4q28.3-qter) (Celle et al., 2000). The brain is uniquely susceptible to chromosomal imbalance, and Tyshchenko et al. (2009) have constructed a map. We have undertaken similar phenotype mapping studies with respect to kidney defects (Amor et al., 2003), and to epilepsy (Singh et al., 2002a), hypothesizing that the chromosome regions thus illuminated may serve as candidate regions for the discovery of renal and epilepsy genes.

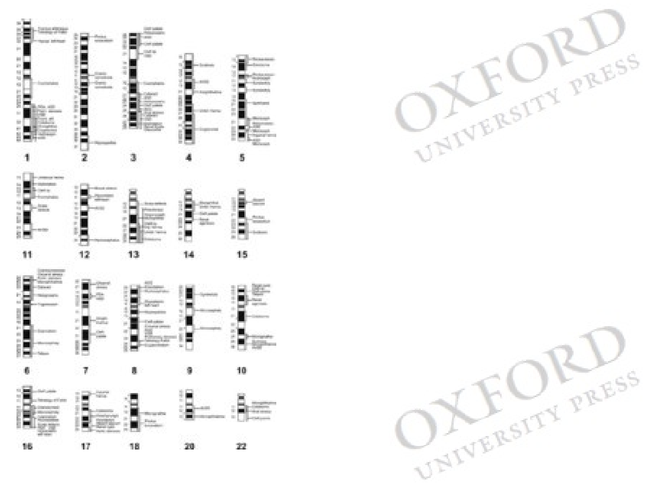




Figure 3–11
A duplication-malformation correlation map. Some chromosomal regions, in the duplicated state, are particularly associated with certain types of malformation. Presumably, these regions harbor genes that have roles in the formation of these particular organs. Other regions (including all of chromosome 19) are unrepresented, and some of these may contain “triplo-lethal genes.” ACC, agenesis of the corpus callosum; ASD, atrial septal defect; AVSD, atrioventricular septal defect; PDA, patent ductus arteriosus; VSD, ventricular septal defect. (From C Brewer et al., 1999, A chromosomal duplication map of malformations: regions of suspected haplo- and triplolethality—and tolerance of segmental aneuploidy—in humans, *American Journal of Human Genetics* 64:1702–1708. Courtesy D. R. FitzPatrick; reproduced with the permission of the University of Chicago Press.) A similar map has been drawn for deletions (Brewer et al., 1998).

The application of microarray analysis enables a finer focus to be taken. Catelani et al. (2009) have looked for “microimbalances” in children with syndromic deafness, anticipating that these might point the way to the discovery of hearing genes (Table 3–2). Kirchhoff et al. (2009) describe correlations in the distal half of chromosome 13 on the basis of microarray analysis, associating particular traits with certain “microsegments” (Fig. 19–2). Not that a one-to-one connection between a deleted/duplicated segment and a specific trait can necessarily be drawn; and for example, we have proposed that the particular nervous system malformation of periventricular nodular heterotopia might be an epiphenomenon accompanying a number of microdeletion syndromes, rather than the direct consequence of specific segmental imbalances (van Kogelenberg et al., 2010).

Table 3–2. An Example of the Precision That Microarray Analysis Allows, and How the Data May Be Presented

	GENOMIC POSITION, MB FROM PTER			
	PROXIMAL BREAKPOINT	DISTAL BREAKPOINT	MINIMUM SIZE (Mb)	ORIGIN
del(1)(q23.3q25.2)	160.19 … **161.21**	**175.27** … 175.56	14.06	de novo
dup(2)(q22.2q23.3)	143.67 … **144.59**	**150.78** … 150.86	6.19	de novo
dup(6)(p25.2p25.3)	2.98 … **2.86**	**1.26** … 1.32	1.60	de novo
del(11)(q13.2q13.4)	67.55 … **68.07**	**70.31** … 70.60	2.24	de novo
dup(2)(q12.3q12.3)	107.03 … **108.13**	**108.34** … 108.50	0.21	maternal
del(4)(q23q24)	101.15 … **101.51**	**101.96** … 103.22	0.46	paternal
del(7)(q31.1q31.1)	109.93 … **110.06**	**110.31** … 110.39	0.25	maternal
del(15)(q15.3q15.3)	41.41 … **41.68**	**41.85** … 42.76	0.17	maternal

Notes: Measurements in boldface indicate breakpoints for the minimum determined size of the deletion/duplication; nonboldface indicates maximum size.

These “microimbalances” were identified in a group of children with syndromic hearing loss, assessed at the Genetic Counseling Service of the University of São Paulo, whose standard karyotypes had been interpreted as normal (Catelani et al., 2009). The deleted/duplicated microsegments in the de novo cases presumably included genes whose imbalance contributed to the other phenotypic traits manifested by these children, but which might also have included a “hearing gene,” for which a dosage imbalance could have compromised function of the gene product. For example, the deletion of chromosome 1 included *DFNA7*, a gene which is the basis of one form of dominantly inherited nonsyndromic deafness, and *DFNA7* haplo-insufficiency might have contributed to the child's profound deafness. The case in those having inherited the imbalance from a normal parent is less secure; these could be normal copy number variants.

For the most part, the clinical states due to chromosomal imbalance are fixed and static. Structural defects such as a cardiac septal defect, or facial dysmorphism, are not progressive (although they may be evolving) conditions: they were established during embryogenesis and fetal development, and, in essence, will stay that way. They may, of course, set the stage for consequential progressive change, such as a urinary tract defect that has back-pressure effects upon a kidney, affecting renal function; but this is a secondary factor. The brain, the most vulnerable organ, is similarly fixed in terms of its underlying anatomy, and chromosome disorders would not typically be described as neurodegenerative. The most notable exception to that rule is the long-recognized dementia that typically commences around age 40 in Down syndrome, and which reflects the effects of a triple dose of the amyloid precursor protein gene on chromosome 21, with a gradual accumulation in the brain of the abnormal protein. Other exceptions exist. De Bruijn et al. (2010) describe a de novo apparently balanced translocation in which no genes were lost, but the new position in the genome of a “brain gene” (*LRFNS*) led to its much diminished activity (thus, an epigenetic effect), and this was associated with an actual loss of acquired skills in mid-childhood. The cognitive deterioration in the ring 20 syndrome may reasonably be described as neurodegenerative, albeit that the onset of epilepsy may be a necessary aggravating factor.

Database Resources

Two important hard-copy sources provide information on, first, the clinical features of specific duplications and deficiencies, and second, viability of a particular segment. Schinzel has compiled his *Catalogue of Unbalanced Chromosome Aberrations* (2001), an invaluable resource documenting the clinical phenotype in about 2000 different aneuploid states that are compatible with live birth, and many of which are associated with survival through to early childhood or beyond. Stengel-Rutkowski et al. (1988) have gathered data from 1120 translocation pedigrees, determining, for numerous segments in the partially trisomic and partially monosomic states, the likelihood that a pregnancy would proceed through to live birth (referred to in detail in Chapter 5).

It is an obvious point, but worth restating: the defect in these aneuploid states involves too much or too little of what is *normal* chromosome material. The “third” chromosome in standard trisomy 21 is a perfectly normal chromosome 21, with a perfectly normal complement of chromosome 21 genes. How, therefore, could it be that an additional amount of normal genetic message leads to an abnormal interpretation of that message? This is one of the great remaining unanswered questions of biology, which we touch upon (no more than that) in Chapter 18.

The Sex Chromosomes

Sex chromosome imbalances need to be considered separately. Any X chromosomes in excess of one are genetically inactivated. Thus, indicating the inactivated X in lowercase, normal females are 46,Xx, normal males are 46,XY, Turner females are 45,X, Klinefelter males are 47,XxY, and other X aneuploidies are 47,Xxx, 48,Xxxx, 48,XxYY, 49,XxxxY, 49,Xxxxx. As for the Y chromosome, its active genetic material is confined to only a small segment, these genes being mostly related to sex determination and testicular function. Thus, in spite of the presence of one or more whole X or Y chromosomes in excess in the 47-, 48- and 49-chromosome states, in utero survival remains possible. Indeed, for 47,XXX, 47,XXY, and 47,XYY, survival is apparently quite uncompromised. Gonadal development in X aneuploid males is particularly affected, and intellectual function is jeopardized to a mild or moderate extent in the n ≥47 states in both sexes. 45,X has a high in utero lethality, although the small fraction surviving to term as females with Turner syndrome show, in contrast, a remarkably mild phenotype.

Phenocopies

Similar phenotypes may flow from different genotypes. “Pseudotrisomy 13” may be an autosomal recessive condition (Amor and Woods, 2000). The expression “DiGeorge syndrome” refers to an ensemble of signs that characterize the 22q11 deletion. Somewhat similar clinical pictures can be seen in deletions of 10p13 and of 4q34.2. Syndromes resembling Silver-Russell syndrome, Prader-Willi syndrome, and Angelman syndrome, but due to other chromosomal imbalances, are described (see Chapter 22).

The Mosaic State

Whether mosaicism matters depends upon which tissue, and how much of that tissue, is abnormal. If a majority of the soma is chromosomally abnormal, the phenotype is likely to be abnormal. If only a tiny fraction of some tissue were involved, in which the aneuploidy would have essentially no effect—if, say, some of the bony tissue of the distal phalanx of the left little toe were trisomic 21, and the rest of the person 46,N—it would never be known. Indeed, as mentioned earlier, it could be everyone has mosaicism, for the most part harmless, in certain tissues or organs. However, in regard to disease, a very minor degree of mosaicism could still be important if a crucial tissue carried the imbalance. An abnormal chromosome confined to tissues of, say, a localized area or cell type in one part of the brain could theoretically cause neurological dysfunction.

(Perhaps twenty-first century technology will devise a “functional cytogenetic MRI scan” that could map out brain regions with an aneuploid chromosomal complement!) Abnormality involving a gonad or part of a gonad (“gonadal mosaicism”) could lead to a child being conceived with that aneuploidy. Mosaicism confined to extraembryonic tissue may be without phenotypic effect, although it certainly causes anxiety if it produces an abnormal test result at prenatal diagnosis. Confined placental mosaicism (CPM) may exist unbeknown in pregnancies producing normal infants, as Lestou et al. (2000) showed in a study of 100 placentas, with five revealing CPM for trisomies 2, 4, 12, 13, and 18. Mosaicism may frequently be observed at the IVF laboratory in the early cleavage embryo, and of spectacular degree, with different cells having different aneuploidies; a state of affairs that becomes very relevant in preimplantation genetic diagnosis (Chapter 26).

Mosaicism for a Full Aneuploidy

As a general principle, an individual with an aneuploid line in only some tissues is likely to have a less severe but qualitatively similar phenotype to someone with the nonmosaic aneuploidy. The ascertainment of these individuals is biased: those with a more obvious phenotypic defect are, naturally, more likely to be detected. Mosaic Down syndrome—46,N/47,+21—can be less obvious than standard trisomy 21, and with a lesser compromise of intellectual function. The existence of 46,N cells in some of the brain tissue presumably has a moderating effect. Some aneuploidies can only, or almost only, exist in the mosaic state, the nonmosaic form being lethal in utero. Examples of this are 46,N/46,+8 and 46,N/47,+9 mosaicism. If the distribution of the aneuploid cell line is asymmetric, body shape may be asymmetric, generally with the hypoplasia in regions of aneuploidy. De Ravel et al. (2001) described hemifacial microsomia (one side of the face being underdeveloped) and other body asymmetry in two children with autosomal mosaicism, one for trisomy 9 and the other trisomy 22. The child with 47,XY,+22/46,XY had 9/10 cells +22 on skin fibroblasts from the arm on the right (underdeveloped) side, compared with 5/11 on the left arm (blood karyotype was 46,XY). Molecular analysis supported there having been a postzygotic anaphase lag that had produced the 46,XY line from an initially 47,XY,+22 conception. A surprising case is that of Greally et al. (1996): a child with mosaic trisomy 16, a cardiac malformation, and otherwise (barring a unilateral simian crease) not dysmorphic, and her neurodevelopmental progress has been quite normal. One might suppose (but could not prove) that the trisomic cell line was somewhat confined in distribution and excluded the brain.

Mosaicism excluding the bone marrow will give a normal blood karyotype, and vice versa mosaicism confined to marrow would be seen on routine peripheral blood analysis, but not on other samplings; mosaic trisomy 8 may provide examples in both directions. Examples of presumed very low-level trisomy mosaicism have come to light through prenatal diagnosis, such as trisomy 13 mosaicism in an apparently normal child with 1 cell out of 400 on cord blood (Delatycki et al., 1998). In sex chromosome mosaicism, fertility can exist when otherwise infertility is the rule: for example, in “formes frustes” of Turner syndrome with 45,X/46,XX and of Klinefelter syndrome with 46,XY/47,XXY.

Mosaicism for a Structural Rearrangement

We reviewed a three-decade experience in New Zealand, and only 12 cases of mosaicism for a structural rearrangement had ever been recognized, of which at least 3 were regarded as balanced, and 8 presumably unbalanced. This equated, crudely, to an incidence of around one case per year per 1,000,000 population (Gardner et al., 1994). This is likely to be a considerable underestimate. Leegte et al. (1998) reviewed the literature and could record 29 cases of mosaicism for a *balanced* reciprocal translocation. This state, presumably, would be without any phenotypic effect. The only practical implication would be if the mosaicism extended into the gonad, an example of which is provided by Shapira et al. (1997c). A man, himself quite normal, was mosaic for a pericentric inversion, 46,XY/46,XY,inv(9)(p24q34.1), and he had a child with a recombinant chromosome that had caused a deletion 9p/duplication 9q syndrome. Some cases of “balanced” translocation mosaicism will have been ascertained through the phenotypic abnormality of a proband, and it is arguable whether the rearrangement had caused the abnormality or was coincidental (Aughton et al., 1993).

With an *unbalanced* karyotype, the broad rule applies that the mosaic form is likely to be less severe than the nonmosaic. Pigmentary skin anomaly is a notable and clinically useful phenotypic trait that can characterize this type of unbalanced mosaicism, the important categories being hypomelanosis of Ito, linear and whorled nevoid hypermelanosis, and “phylloid” (leaf-like) pigmentary disturbance (Verghese et al., 1999; Riberio Noce et al., 2001). The distribution of the abnormal cells in hypomelanosis of Ito, and thus of dyspigmentation, follows the lines of Blaschko, and Magenis et al. (1999) use the expression “Blaschkolinear malformation complex.” Niessen et al. (2005) studied in some detail a girl with three shades of skin pigmentation—hypopigmented, normally pigmented, and hyperpigmented (“cutis tricolor”)—following the lines of Blaschko. She karyotyped 45,X on blood, and 47,XX,+7 on skin biopsied from the darker skin. Asymmetry is a further clinical pointer (Woods et al., 1994). “Functional mosaicism” for a structural rearrangement is exemplified by the X-autosome translocation in which different regions of the body have differing ratios of inactivation of the translocation and the normal X, and this, also, can lead to hypomelanosis of Ito (Hatchwell et al., 1996).

An interesting category of mosaicism for a structural rearrangement is that in which two lines of opposite imbalance coexist, with or without a normal cell line as well. Here, the error must have happened at a very early stage, and quite possibly, in those cases lacking a normal cell line, at the very first mitosis of the zygote. Such a case is described in Morales et al. (2007a), who analyzed a boy with the karyotype at birth of 46,XY,del(7q)/46,XY,dup(7q), although by age 12–14 months, the deletion cell line had disappeared, at least from blood and exfoliated urinary epithelial cells. Presumably, the karyotype at conception was 46,XY; but then the two chromosome 7 homologs underwent an unequal exchange of q21.1–31.3 material, generating, in the two daughter cells from the first mitosis, the deletion and duplication lineages. If, say, the error occurred one division later, at one of the two second mitotic divisions, a normal cell line might be retained, according to which progeny cells then came to comprise the inner cell mass.

Tissue Sampling in the Detection of Mosaicism.

As already noted, mosaicism can, in theory, be very widespread, and the distribution of the different cell lines can vary considerably. Analysis of tissues other than blood can clarify the picture: readily accessible tissues such as buccal mucosal cells and urinary epithelial cells, and infrequently available material from posttermination or postmortem studies. Kingston et al. (1993) described a fetal study in which several tissues taken posttermination had various fractions of mosaicism for an additional abnormal chromosome, including 88% of brain cells, while only 3% of amniotic fluid cells and no cells from a sample of fetal blood had the abnormal chromosome. Reddy et al. (1999) studied a retarded woman with mosaicism for an “add(3),” whose blood karyotype proved to be 46,XX,der(3)t(3;14)(q29;q31)/46,XX. Using a 14q subtelomeric probe, they could show that 86% of buccal cells showed three FISH signals, and so contained the der(3), while the 14% of cells with two signals were normal. (In fact, this ratio was very similar to that of the peripheral blood, which was 83:17.)

Gonadal (and Somatic-Gonadal) Mosaicism

The classical view is that gametes with a chromosomal abnormality are typically produced by 46,N parents, whose gonads are chromosomally normal. The abnormality is presumed to have arisen at meiosis and to have affected only the gamete(s) arising from that single meiosis. If, however, an abnormality had arisen during formation of a germ cell *prior* to the onset of meiosis, an abnormal cell line can become established and occupy a part of the gonad or gonads. This is gonadal mosaicism.

Gonadal mosaicism is suspected upon the observation of a chromosomally normal couple (on blood testing) having had two children with the same abnormal karyotype. Molecular analysis can allow an inference of who is the carrier parent, such as Tosca et al. (2010) show in the family study of two children with a dup(4)(q22.2q32.3), in which the microsatellite pattern indicated a maternal origin. Definitive proof is provided by analysis of gametes. For example, in a case that had come to notice through an IVF clinic, Somprasit et al. (2004) report a couple having had a 21q duplication in two embryos subjected to preimplantation genetic diagnosis (PGD), and then, using FISH, showed the same duplication in 6.6% of 1002 of the father's sperm. The abnormality was not present in his blood. The direct testing of ovarian tissue is noted below.

Cells destined to give rise to gametocytes originate from the yolk sac in early embryogenesis and migrate to the gonadal ridge on the dorsal wall of the abdominal cavity, where, along with the supporting cells, they come to comprise the tissue of the gonad. In doing so, gametocytes must replicate many times, going through about 30 cycles of division. Thirty cycles produces 2^{30} (about 1,000,000,000) progeny cells, and the potential for error exists at each cell division contributing to this population. These errors could be nondisjunctions or the production of structural rearrangements. Edwards (1989) offers a startling insight into the actuality of gonadal mosaicism. He points out that, in the male, the total length of seminiferous tubule is about 1 kilometer. If a mutation were to occur in a spermatogonium in, say, the twentieth cycle of division, its progeny would then go

through 10 more cycles and comprise 2^{10} (about 1,000) cells. This would be only a millionth (1000/1,000,000,000) of the 1 km of tubule—a mere 1 mm. So a man mosaic in such a way would have a risk of only 1 in a million to father a conception with this particular abnormality. From similar reasoning, a defect arising at the tenth cycle could affect 1 m of tubule, and thus carry a risk of 1 in 1000. Oögonia need go through a lesser number of cycles, but the same principles broadly apply.

If the abnormality arose in embryogenesis prior to the differentiation of the germ cell line, the soma may also be involved: this is *somatic-gonadal mosaicism*. Sachs et al. (1990) demonstrated ovarian mosaicism by direct gonadal samplings in a woman who had had one Down syndrome child and three other trisomic 21 pregnancies; she had already been shown as a (very low-level) somatic mosaic, her blood karyotype 46,N[97%]/47,+21[3%]. Tissue cultured from ovarian biopsies showed almost half the cells in each ovary to be 47,XX,+21. Other such cases are mentioned on p. 282. Hultén et al. (2008), from an analysis of fetal ovaries, suggest that ovarian mosaicism may be a great deal more frequent than has been recognized, and they make the challenging, indeed startling statement that “parental aneuploidy gonadal mosaicism may be the major underlying reason for trisomy 21 conceptions” (and see earlier discussion).

Gonadal mosaicism can also arise due to “correction” of an initially trisomic conception (Fig. **3–8**). The classic example is mosaic trisomy 21, in which a 47,+21 embryo discards the extra chromosome in a cell which goes on to produce most tissue lineages, but not in the cell destined to give rise to gonadal tissue (Kovaleva, 2010). The female embryo may engage this process more efficiently than the male. The clinical observation supporting the interpretation is that the mosaic mothers (who presented due to having had a nonmosaic DS child) are of a typical maternal age range, whereas *their* mothers—the grandmothers of the DS children—were of older maternal age at the time their daughters had been born.

Figure **3–12** shows an example of somatic-gonadal mosaicism for a structural rearrangement. The index case was identified with a small intrachromosomal del(1) at routine prenatal diagnosis. The father was mosaic for this deletion, in 20% of lymphocytes. Of his two other children, one had normal chromosomes, and the other had the same deletion. The father is phenotypically normal, and the older child with the deletion has an IQ in the low normal range. A similar circumstance is recorded in Fan et al. (2001): a university-educated man working as a financial planner, having the blood karyotype 46,XY[24]/46,XY,dup(8)(p21.3p23.1)[6], fathered two children with 46,XX,dup(8)(p21.3p23.1). These girls had poor language development, clumsy motor abilities, and minor facial dysmorphism. If the proportion of abnormal cells in the mosaic parent was higher or differently distributed, that parent may manifest some signs of the partial aneuploid state. The father reported in Kennedy et al. (2001) had a dup(8)(p23.1p23.1) in mosaic state, in the ratio normal:duplication of 17:8 on blood sampling, and he himself had a heart defect, as did his nonmosaic dup(8) daughter. Her defect was, however, rather more severe than her father's: she had a fairly complex malformation, including a right-sided aortic arch, while he had only a right-sided arch. Notably, the daughter was described as “achieving top grades in school,” a very unusual phenotypic commentary in a child with a nonmosaic chromosome duplication.

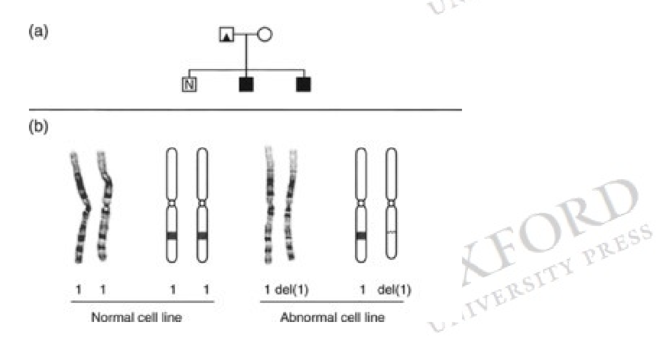




Figure 3–12
A family exemplifying somatic-gonadal mosaicism. (*a*) Pedigree. The father had the mosaic karyotype 46,XY,del(1)(q25q31.2)[16]/46,XY[4] on lymphocyte study. Two children have the del(1)(q25q31.2) in nonmosaic state. The family was ascertained following routine prenatal diagnosis. The older sibling's development was judged, at age 5 years, to be in the low average range; height, weight, and head circumference were in the range 20–25th centiles. The father worked as an electrician. (*b*) Partial karyotype showing the father's two cell lines: two normal no. 1 chromosomes, and one normal and one deleted chromosome 1. The segment 1q25-q31.2 is shown crosshatched. (Case of G. Dawson.)

Placental and Amniotic Fluid Mosaicism

About 1%–2% of placentas can have a different chromosomal constitution from that of the embryo, with usually the embryo being normal and the placenta trisomic. This is “confined placental mosaicism.” Thus, in 1%–2% of chorionic villus sampling (placental biopsy) there will be a potentially misleading result. Fortunately, these uncommon instances can, as a rule, be recognized as such, although not without causing some anxiety at the time. In a few confined placental aneuploidies, the function of the placenta may be compromised, and fetal well-being may be affected.

Infrequently, true mosaicism is recognized at amniocentesis. Occasional cells with a chromosomal abnormality, if they are solitary or involving a single clone, are generally regarded as having arisen in vitro (“artifactual mosaicism”). At least most of the time, this is probably the correct interpretation. We consider placental and amniotic fluid cell mosaicism in detail in Chapter 27.

Qualitative Imbalance

The idea that abnormality could be due to unequal parental contributions of an overall correct amount of chromosome material seemed most remarkable in 1980 when Engel first made the suggestion and coined the expression “uniparental disomy.” It has come to be accepted fact. The two disorders that, par excellence, exemplify the concept of qualitative imbalance are Prader-Willi syndrome (PWS) and Angelman syndrome (AS). The concept of genomic imprinting, discussed earlier, is central to an understanding of the etiology. Each syndrome is due to the nonexpression of different (but neighboring) segments within the proximal long arm of chromosome 15. A “*PWS critical region*” is normally expressed from only one chromosome, in this case the paternally originating chromosome. The maternal-originating region is normally inactive and alleles in this region are not transcribed. Thus, there is a “functional monoallelism.” If the paternal PWS region is absent, the maternal one cannot “fill the gap,” and the consequential functional nullisomy is the root cause of PWS. An “*AS critical region*” exists, lying just a little distal from the PW region. Likewise, it needs only monoallelic expression for normal phenotypic function. In this case, the maternal region is active, and the paternal region, having been imprinted, is inactive. If the maternal region is absent, there can be no genetic activity, and this causes the AS phenotype.

Absence of the paternal PWS region or maternal AS region can flow from two major mechanisms. First, in uniparental disomy (UPD), one parent fails to contribute a chromosome 15; and the “correcting” presence of two copies from the other parent cannot restore a proper balance. This can be heterodisomy (the two homologs being different), or isodisomy (they are identical). Second, there can be a deletion within proximal 15q that removes a segment of chromatin containing the PWS and AS regions. These issues are dealt with in some detail in Chapter 22.

As the imprinting story has evolved, it has emerged that several, indeed most chromosomes, appear not to be subject to imprinting. For these chromosomes, and with both homologs equally genetically active, regardless of the parent of origin, UPD will have no untoward effect. Only if the UPD-contributing parent should happen to be heterozygous for a recessive gene, and if this is the isodisomy category of UPD, will the child be affected, displaying the condition concerned, due to homozygosity (“isohomozygosity”) for that recessive gene. Rare instances of this scenario are known.

Similar considerations may apply in the trisomies. Naturally, one parent must have contributed more than one homolog. Considering the example of Down syndrome, does the parent from whom the disomic gamete came contribute two different chromosome 21s? In other words, does the child inherit a chromosome 21 from three of the grandparents —"heterotrisomy"? Or does the parent contribute two identical (isodisomic) chromosome 21s? Whether phenotypic differences may flow from these different possibilities is uncertain, although Baptista et al. (2000) suggest that heterotrisomy 21 may, of itself, convey a greater risk for a specific heart malformation, ventricular septal defect, speculatively due to a damaging interaction of three subtly different protein products from a 21q "heart locus."

Uniparental disomy for the entire chromosome set—"uniparental diploidy"—has a devastating effect on development. If a conceptus has lost its maternal complement, and the paternal complement is doubled, embryonic development arrests, leaving only grossly abnormal chorionic villi comprising the pregnancy. This is a hydatidiform mole (p. 394). If a 46,XX ovum at meiosis I attempts a parthenogenetic development, a grossly disorganized mass of embryonic tissue results: an ovarian teratoma. If a triple set of chromosomes (triploidy) is present at conception, there is either a diploid maternal set plus a haploid paternal set, or vice versa. These different parental origins determine very different abnormal fetal and placental phenotypes (p. 287).

Segmental Uniparental Disomy

A mitotic mechanism that can lead to functional imbalance, if the segments exchanged are in a region subject to imprinting, is somatic recombination. The first shown example of this causing a dysmorphic syndrome is the segmental paternal uniparental disomy for 11p that underlies some Beckwith-Wiedemann syndrome, 11p being a segment that is normally maternally imprinted. In the partial UPD(pat) cell line, this segment will now be expressing biallelically at distal 11p, instead of the normal monoallelic expression. The asymmetry of body growth in this syndrome reflects the distribution of two cell lineages: the normal biparental disomic line, and the functionally imbalanced UPD(pat) line.

Sporadic and Recurrent Abnormalities

Chromosomally normal parents can produce abnormal gametes by nondisjunction, or by one of the other mutational mechanisms we have discussed earlier. The combination of factors that causes these defects in an individual case is unknown. No convincing case has ever been made for the agency of diet, illness, chemical exposure, or "lifestyle factors" in maternal chromosome 21 meiotic nondisjunction (Chapter 28), nor is there much support from epidemiological studies (Chapter 24). Noting the similarity of Down syndrome prevalence rates worldwide, Carothers et al. (1999) comment that "the totality of published data could well be consistent with no real variation at all, and [this] might explain why a search for environmental factors associated with Down syndrome has been so unproductive." The maternal age effect is of course important, indeed central, and any search for causes of chromosomal aneuploidy must take it into account. A plausible view is that there is a natural degeneration of the oöcyte, as we discussed earlier, and with reference to Figure **3–7**. Simply put, eggs get older, and they show their age. (But we do not ignore the view of Hultén et al. (2008) that maternal gonadal mosaicism, with delayed maturation of the trisomic 21 oöcytes, is an alternative explanation.)

Chromosomes are plastic, dynamic entities, and cell division is a complex mechanical process; and these qualities alone may suffice to endow the vulnerability that causes human aneuploidy and rearrangement. Given the assumption that all persons with intact gametogenesis are capable of producing an abnormal gamete, one view is that it may simply be that a certain background abnormality rate is intrinsic to the human species, and it is a chance matter whether this or that couple will have the misfortune to conceive the abnormality which, inevitably, someone has to bear.

Parental Predisposition to Nondisjunction?

An alternative view is that some 46,XX and 46,XY people are more prone than others to produce chromosomally unbalanced gametes. An intrinsic fault, or at least a vulnerability, in the mechanism of chromosome distribution at cell division could be the basis of the rare examples of recurring defects. The synaptonemal complex gene *SYPC3* has been mentioned earlier; another candidate is the mismatch repair (MMR) gene, with particular reference to *MLH1* (otherwise familiar to the counselor in hereditary nonpolyposis colon cancer) and *MLH3*, and the related meiosis genes *MSH4* and *MSH5* (Baarends et al., 2001; Lenzi et al., 2005; Terribas et al., 2010). Given the complexity of the apparatus and process of cell division, it is logical that error-causing mutants in the controlling genes (regardless of whether this might include any of the aforementioned) would exist. Whether there might be milder alleles at postulated cell-division loci, which could more widely be the cause of occasional nondisjunction, remains a matter for speculation. Nevertheless, a geneticist could scarcely ignore that there might exist subtle genetic variation potentially setting the stage for nondisjunction.

A Note on the Diagrams.

Following the progress of rearranged chromosomes during meiosis is not easy, so we have taken some liberties in simplifying the diagrams. Most of these diagrams depict the synapsing chromosomes at meiosis with just one chromatid; of course, the chromosome has actually replicated at this point and exists as a double chromatid entity (Fig. **3–13**).

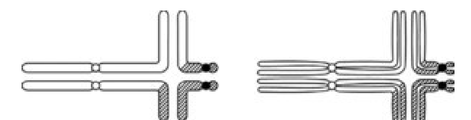


Figure 3–13
Chromosomes at synapsis exist as double-chromatid structures (e.g., the reciprocal translocation quadrivalent at right). But, for simplicity, we generally represent them with just the one chromatid (*left*).

Notes:

1 The law of independent assortment: During gamete formation the segregation of the alleles of one allelic pair is independent of the segregation of the alleles of another allelic pair. The exception: If two loci are close together—"linked"—on the same chromosome.

2 Cytoplasm contains the mitochondria, and transmission of mitochondrial DNA is essentially maternal. The mitochondrial genome has been described, somewhat whimsically, as chromosome 25, or the M chromosome. In not otherwise referring to this "chromosome," we are not seeking to deny its importance or interest!

3 Since, at the level of the chromatid, there are four elements, the word *tetrad* can also be used in this setting. In one sense, the cell at this stage of the cycle has 23 × 4 = 92 chromatids. At the molecular level, the number of single DNA strands is eight.

4 Somewhat confusingly, some writers use this word to mean "gonadal-somatic," when referring to mosaicism for a mendelian gene.

5 For the record, the chimera of classical mythology was "in the forepart a lion, in the hinder a serpent, and in the midst a goat."

6 Somewhat stretching the analogy, Bianchi (2000) makes the intriguing suggestion that, due to the retention and persistence of fetal blood cells following delivery, every mother is, in a sense, a chimera ("microchimerism").

7 Note that disjunction is a normal process, and nondisjunction is not; there is no such word as *dysjunction*.

8 If the homologs had never joined together, then they could not disjoin. In that sense, *nonconjugation* might be a more accurate word than *nondisjunction*, a point Sturtevant

and Beadle (1962) made many years ago. Following Angell, *predivision* may be a proper word. Nonetheless, *nondisjunction* is well entrenched in the genetic lexicon, and its general meaning of “the inclusion of both daughter chromosomes in the same nucleus, by whatever mechanism” (Miller and Therman, 2001) is well accepted.

9 A very rare example of autosomal monosomy/disomy/trisomy mosaicism was identified in the abnormal baby reported in Stefanou et al. (2006), mentioned earlier. Only 1 cell in 200 on blood showed 47,XY,+20, and disomy demonstrated on buccal mucosal FISH and skin fibroblast analysis, but 39/50 cells from urinary sediment were monosomic.

10 Dr. Maj Hultén was the second person in the world to know that the human chromosome number was 46 (Harper, 2006).

11 But exceptions exist, and around 5% of autosomal genes are randomly expressed from only one or other parental allele (Gimelbrant et al., 2007).







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Deriving and Using a Risk Figure

Chapter: Deriving and Using a Risk Figure
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RISK IS A CENTRAL CONCEPT in genetic counseling. By *risk*, we mean the probability that a particular event will happen. Probability is conventionally measured with a number ranging from 0 to 1. A probability (*p*) of zero means never, and a probability of 1 means always. For two or more mutually exclusive possible outcomes, the individual probabilities sum to 1.0 (or 100%). Thus, someone who is a heterozygote for a particular rearrangement might, in any given pregnancy, have a probability of 0.10 (10%) of having an abnormal child and a probability of 0.90 (90%) of having a normal child. We may speak in terms of risks of *re*currence or of *oc*currence: the probability that an event will happen again, or that it will happen for the first time. Risk can also be presented as *odds*: the ratio of two mutually exclusive probabilities. The odds for the preceding hypothetical heterozygote would be 9:1 in favor of a normal child.

The work *risk* has two important meanings in the English language. First, there is the scientific sense of probability that we already discussed. Second, as most people use the word, it conveys a sense of exposure to danger. Our hypothetical heterozygote runs the risk that an unfortunate outcome may occur (an abnormal child, or an abnormal result at prenatal diagnosis). In the genetic counseling clinic these meanings of risk coalesce in some ways, to which the counselor needs to be sensitive. We might instead use such everyday words as *chance* or *likelihood*, which have no negative connotation, to refer to the fortunate outcome of normality. The words *fortunate* and *unfortunate* are also chosen deliberately: the wanted or the unwanted event will occur entirely by chance, analogous to tossing a coin, throwing a dice, or being dealt a card.

Different Types of Risk Figure

Geneticists arrive at risk figures in a number of ways (Harper, 2010), two of which have particular application to cytogenetics.

1. *Empiric risks*. In the great majority of chromosomal situations, no clear theory exists from which the risk can be derived, and one must observe what has happened previously in (as far as one can judge) the same situation in other families and make an extrapolation to the family in question. Empiric risks thus appeal to experience, and they only *estimate* the intrinsic, true probability. The data may be available in the literature record or in specific databases; or the counselor may need to derive a "private estimate" from an analysis of the client's family. The risk estimate has a greater or lesser degree of precision, depending on how much data have been accumulated upon which the estimate is based.
2. *Mendelian risks*. If a clear model of inheritance is known, risk figures derived by reference to that theory may be used. In practice, only Mendel's law of segregation is applied in this context. When a pair of homologous chromosomes segregates at meiosis, it is a random matter which chromosome enters the gamete that will produce the conceptus. Each has an equal chance: a probability of 0.5. As an example, the X chromosomes in the fragile X heterozygote, the normal X and the fragile X, display 1:1 segregation. In the microarray era, the 1:1 segregation of copy number variants becomes of relevance. This is assumed to be a *true* risk, not an estimate: it is 0.5 exactly.

Consider, for example, the common situation of a young couple having had a child with Down syndrome. Nothing is known about nondisjunction that could provide a theoretical model on which to base a recurrence risk figure. We therefore use empiric data, that is, information obtained from surveying large numbers of other such families. It may be observed, for example, that in these families about 1 pregnancy in 100, subsequent to the index case of Down syndrome, produced another child with Down syndrome. Formally expressed, this is a segregation analysis. From this rate of 1/100 we can derive a risk figure of 1%, which we then have as the basis for advising patients. (Actually, it is not quite as straightforward as this in Down syndrome; see Chapter 18.) Likewise for the circumstance of the parent heterozygous for a chromosomal rearrangement, the counselor can consult data that have been accumulated by workers in the field, foremost among whom, in respect of reciprocal translocations, are Stengel-Rutkowski et al. (1988) and Cohen et al. (1992, 1994), and more recently, Midro et al. (2000). Since almost all reciprocal translocations are unique to one family, it is not necessarily simple to estimate a figure for a family with a "new" translocation, but an attempt can be made (see Chapter 5). On the other hand, for the Robertsonian translocations, each type of which can generally be regarded as the same between families, extrapolation of risk figures from historical data to a current family is usually valid.

Hook and Cross (1982) note the importance of distinguishing between the *rate* (which may be thought of as "past tense") and the *risk* (which is "future tense"). They emphasize that, while geneticists routinely extrapolate from rates in one population at one point in time, and may use these figures as risk estimates in another population and certainly at a later point in time, they should be on their guard for any evidence that a condition varies with time, geography, or ethnicity. But actually, there is little indication that any important variation exists: chromosomal biology appears to be rather consistent throughout the human race (p. 406).

Doing a Segregation Analysis

Segregation analysis is essentially a simple exercise. A farmer who surveys a flock of newborn lambs and notes that 3 are black and 97 are white has done a segregation analysis. In human cytogenetic segregation analysis, the exercise involves looking at a (preferably large) number of offspring of a particular category of parent: parents who carry some particular chromosome rearrangement or those who have had a child with a chromosomal abnormality, they themselves being karyotypically normal. The proportion

of these parents' children who are abnormal is noted (say, 3 out of 100), and this datum serves as the point estimate of the recurrence risk (thus, 3%).

Although segregation analysis is simple in principle, there are potential pitfalls in its application, the most important of which is *ascertainment bias*. We will deal with this problem only briefly. It is important that the counselor know of ascertainment bias and recognize whether it has been accounted for in the published works consulted. But it is not necessary to understand the complex and sophisticated mechanics of segregation analysis in detail. The reader wishing fuller instruction is referred to Murphy and Chase (1975), Emery (1986), and Stene and Stengel-Rutkowski (1988). The classic example of ascertainment bias is that of the analysis of the sex ratio in sibships of military recruits in World War I. Adding up the numbers of brothers and sisters, there was a marked excess of males. But of course (in 1914–1918) the recruit himself had to be male. Once he was excluded from the total in each sibship, the overall sex ratio was normal, namely, 1.0. Likewise, in a cytogenetic segregation analysis, the individual whose abnormality brought the family to attention—the proband—is excluded from the calculation. That person *had* to be abnormal. Furthermore, that individual's carrier parent, grandparent, and so on in a direct vertical line had to be (almost always) phenotypically normal to have been a parent. These individuals must also be excluded from an analysis of their own sibship, if that generation is available for study. Other sibships may be included in full.

These manipulations—dropping the proband and the heterozygous direct-line antecedents—are the major steps to be taken to avoid the distorting effects of ascertainment bias. Another potential methodological confounder for the aficionado is *ascertainment probability*. For example, families with more affected members may be more likely to come to medical attention, which would unduly weight the data. There are means to overcome this problem.

Essential to a good analysis is good data, or at least as good as possible. Some retrospective information may be uncertain. In a family translocation study, did a phenotypically abnormal great uncle who died as a child in 1930 have the "family aneuploidy"? (Old photos may be very helpful in this respect.) Some family skeletons may remain in cupboards unopened to the interviewer. Particularly in the follow-up of prenatal diagnosis results, it is important to know the endpoint of data collection of the child and how the data were collected: at birth, or until school age; by formal examination, or by anecdotal report. The investigative zeal, clinical judgment, and personal qualities of the researcher are crucial in getting the right information, and getting it all.

The Derivation of A "Private" Recurrence Risk Figure

We will demonstrate some of the previously noted principles in estimating a private recurrence risk figure for the hypothetical family depicted in Figure **4–1**. Six sibships are available for analysis: one in generation II, two in generation III, and three in generation IV. We determine the segregation ratio in each. It is conventional to form a table with a row for each sibship, noting the numbers of phenotypically normal (carrier, noncarrier, unkaryotyped) and phenotypically abnormal offspring. The figures in parentheses give raw totals in these sibships, but then the proband (IV:4) and his heterozygous antecedents (II:1 and III:1) are excluded from their sibships. Thus, we have the following:

PARENT OF SIBSHIP	PHENOTYPICALLY NORMAL			
	AFFECTED	CARRIER	NON-carrier	UNKARYO-TYPED
I:1	0	1 (2)	2	0
II:1	1	1 (2)	0	2
II:2	0	1	0	1
III:1	2 (3)	0	1	0
III:2	0	1	0	0
III:7	0	0	1	0
Total	3	4	4	3

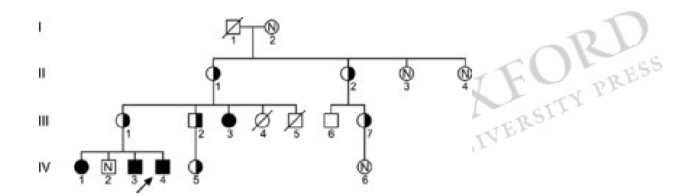


Figure 4–1
Hypothetical pedigree in which a chromosomal rearrangement is segregating. Filled symbol, abnormal individual with unbalanced karyotype; half-filled symbol, balanced carrier; N in symbol = 46,N. The proband is, as is conventional, indicated by an arrow.

(Note, in passing, I:1's heterozygosity must be inferred from his wife's and children's karyotypes. It is a subtle question whether his offspring should properly be included in the analysis, which we will not pursue here). We see that the offspring of heterozygous parents total 14, the proband and the heterozygous antecedents having been excluded. The proportion of abnormal children is 3/14 (0.21). This, then, is a point estimate of the risk for recurrence in a future pregnancy of a heterozygote. The reader should know intuitively that an estimate based on just 14 children is not going to be very precise. And what of children who died in infancy, before the family cytogenetic study has been done? Let us suppose this was the case with III:4 and 5. If there was good evidence for their having been chromosomally abnormal, a better estimate would be 5/14 (0.36).

Genetic Heterogeneity and the Use of Empiric Risk Data

It is not necessarily valid to extrapolate from one family's experience to a prediction for another. Different factors may cause an abnormality in different families. As an obvious example, it would be misleading to "lump" all Down syndrome families to determine a recurrence risk figure. We need to "split" into the different karyotypic classes of standard trisomy, familial translocations, and de novo translocations. The standard trisomic category requires further splitting in terms of maternal age. In a unique case, a woman had three trisomy 21 conceptions and displayed a tendency to produce multiple cells with differing ("variegated") aneuploidies in at least skin, blood, and gonad (Fitzgerald et al., 1986) (and see Chapter 21). She required unique advice. And in reciprocal translocation families, uniqueness is the rule! It is generally reasonable (and often all that is feasible or possible) to apply a risk figure derived from the study of families with a similar, albeit not exactly identical, chromosomal arrangement. But occasionally a family is large enough for a "private" estimate of the recurrence risk to be made from the family itself. This estimate, if it is precise enough (see the later discussion of confidence limits and standard error), is the most valid to offer that family.

Pregnancy Outcomes to which The Risk Figures Refer

With particular reference to the situation of a parent heterozygous for a chromosomal rearrangement, risk figures are generally presented in terms of "the risk that a liveborn child would have a chromosome imbalance related to the parental translocation." The numerator is the number of aneuploid babies, and the denominator the number of all babies. Thus, considering the example of the common t(11;22)(q23;q11) translocation (p. 86), Stengel-Rutkowski et al. (1988) accumulated data on a total of 318 births (the denominator) to carrier parents, of whom, after ascertainment correction, 9 (the numerator) had the 47,+der(22) aneuploidy; and 9/318 gives the risk expressed as a percentage, 2.8%. Separating out mothers and fathers, the respective risk figures are 3.7% (9/241) and <0.7% (0/77). For those choosing prenatal diagnosis, the risk figure of interest relates to the timing of the procedure, generally chorionic villus sampling (usually done at 10–12 weeks) and amniocentesis (15–17 weeks). In other words, they want to know how likely it is they will have to face the actuality of termination. The risk here is likely to be higher (7% in the case of the 11;22 translocation), given that some of the abnormal pregnancies would have spontaneously aborted some time after that period of gestation. Table 4–1 sets out these and other possible ways of considering risk.

Table 4–1. Different Ways of Looking at the Quantum of Reproductive Risk due to a Parent Being a Carrier of a Chromosomal Rearrangement

NUMERATOR	DENOMINATOR
Abnormal liveborn baby	All liveborns
Abnormal liveborn baby	All recognized pregnancies
Abnormal amniocentesis result (early second trimester)	All pregnancies at ~16 weeks
8–14 week miscarriage	All recognized pregnancies
Abnormal embryo on biopsy	All embryos from one IVF procedure
Normal embryo on biopsy	All embryos from one IVF procedure

IVF, in vitro fertilization.

Association: Coincidental or Causal?

The counselor not infrequently encounters the problem of a chromosomal "abnormality" discovered in a phenotypically abnormal individual, but in whose family others—who are quite normal—are then shown to have, apparently, exactly the same rearrangement. Does a genetic risk apply, then, to children of the carrier, to whom the same rearranged chromosome may be transmitted? From classical cytogenetics, the familial paracentric inversion is a good example. In a review of 69 probands, Price et al. (1987) list the phenotypic abnormalities that led to these individuals coming to a chromosome study. There was a collection of various clinical indications, with no consistent pattern (other than that mental retardation was frequent), and several ascertained quite by chance at prenatal diagnosis. By definition, one parent carries the same inversion; and, if the net is widened, often other relatives as well (Groupe de Cytogénéticiens Français, 1986a). In this context, and provided of course that the carrier relatives are phenotypically normal, one would reach the conclusion that the chromosome rearrangement was balanced, with no functional compromise of the genome; and that it was coincidence that led to its discovery (Romain et al., 1983a).

But when some very unusual clinical picture is associated with a paracentric inversion that is rare or previously undescribed (as most inversions are), some writers are skeptical of coincidence and propose a causal link (Urioste et al., 1994a; Fryns et al., 1994). Similarly, Wenger et al. (1995), noting the coincidence of children with an apparently balanced familial translocation, and being phenotypically abnormal, write that "the chance that two rare events in the same individual are unrelated seems unlikely to us." Here, there is a risk of deception due to "Kouska's fallacy"—Kouska was a fictional nineteenth-century philosopher who concluded that the combination of unlikely events that led to his parents meeting was too implausible to believe, and that therefore he himself could not exist (Lubinsky, 1986). As does Lubinsky, we must insist on the point: the proband *had* to be phenotypically abnormal, and the coexistence of a subsequently discovered different abnormal event (the karyotype) need not be seen as necessarily remarkable.

A similar question arises when two rare karyotypes are seen in the same family, or when one individual has more than one aneuploidy. A double aneuploidy such as Klinefelter plus Down syndrome, 48,XXY,+21, could be interpreted as two separately arising nondisjunctions, but each occurring on the basis of the same underlying predisposing factor (such as maternal age). The two conditions occur together more often than the product of the frequency of each singly, which would be consistent with that interpretation. Alternatively, if the XXY component could be shown to reflect a paternal meiotic error, while the trisomy 21 was of maternal origin, then the association could be seen as coincidental. Two different types of abnormality, such as Klinefelter plus Prader-Willi syndrome, a handful of cases of which have been published (Nowaczyk et al., 2004), might also be judged to reflect two unrelated abnormal events, at least for the deletional form of PWS, given that the mechanisms leading to nondisjunction and to deletion are quite different. The prior probability of two abnormal karyotypes coinciding might be a very small figure (1/2000 × 1/15,000 = 1/30,000,000 in the foregoing example); but recalling that the range of abnormal karyotypes is very wide, it should not necessarily be seen as reflecting some extraordinary predisposition when two abnormalities are diagnosed in the one individual or family. Coincidences do happen; and interesting coincidences are publishable (Schneider et al., 2004).

In the microarray era, the matter of copy number variants (CNVs) brings the question of causality into a sharp focus, albeit that some of the answers may be less than sharp! As discussed in Chapter 3, a small molecular duplication, for example, that might at first sight seem to be a reasonable candidate as the explanation for a child's abnormal phenotype, fades in significance when the same observation is made on the DNA sample from a parent. And yet, in the complexity that CNVs present, there may remain a possibility that such a duplication could contribute to abnormality, when existing on a different genetic background. In other words, a particular CNV may be nonpenetrant in a parent, but penetrant in the child; a concept that hitherto has had little relevance in clinical cytogenetics. We can expect that CNV associations, and their causing or not of abnormality, will continue to be an active area of study (and see Chapter 17).

Presentation of a Risk Figure

A risk figure is a probability statement, and it should be presented as such to the counselee in everyday language—for example: "there is a 50/50 chance for such and such an event"; "the risk for such and such to happen is around 1 chance in 10." The raw probability figure may not of itself be sufficient, and it is a test of the counselor's skill to interpret figures so as to provide empathic guidance rather than presumptuous direction. Loaded interpretative comments such as "The risk is quite high that …" or "There is only a small chance that …" should be used with great care. The perception of a risk figure as high or low may vary greatly according to an individual's personality and life experiences and the way he or she uses the language of numbers; the very act of discussing the risk may help the client see it in a less threatening light (Kessler and Levine, 1987). Some counselors use diagrams with cartoons showing a crowd of 100 people, with the risk fraction shown in a different color.

Dealing with risk advice in a pregnancy, in particular, can be anxiety inducing. Nagle et al. (2009) examined the views of 294 Australian mothers in the postpartum period and recorded preferences for how these women felt, in retrospect, that a risk of having a child with Down syndrome might best have been conveyed. The choices were as

follows, with the fractions of the women choosing each category shown in brackets:

(1) As a number in percentage, such as “1%” or “0.05%”	[13%]
(2) In words such as “no increased risk” or “increased risk”	[13%]
(3) As numbers such as “1 in 10” or “1 in 1000”	[37%]
(4) In words such as “high risk” or “low risk”	[19%]
(5) Other (please specify)	[0%]
A combination of the above	[18%]



None of these stood out as an obvious best, to help the counselor decide on the most appropriate approach. People are different!

And people can see to the same risk from different positions. For example, older women having an increased age-related risk (say, 1 in 100) for a child with Down syndrome may decide against an amniocentesis if a maternal serum screening test gives a risk (say, 1 in 200) that is above the cutoff for access to amniocentesis (1 in 250) but lower than their “starting figure”; whereas a younger woman with an age-related risk of, say, 1 in 500 is likely to opt for amniocentesis if she were to have the same 1 in 200 result from the screening test (Beekhuis et al., 1994).

Precision of the Risk Figure

As noted earlier, theoretical risk figures are true and empiric risk figures are estimates; the former are exact, and the latter are not. For an empiric figure we have a point estimate (e.g., 10%) and a likely range (e.g., 5%–15%) of where the risk actually is. The more data that have been gathered, the more accurate the estimate and the narrower the likely range; and the more confidently, therefore, can the counselor present the figure. The likely range can be measured in different ways. The standard error, which formally measures the precision of the estimate, can be used to give a sense of the region within which the true risk can realistically be considered to lie. The 95% confidence limits define the broad range that very probably ($p = 0.95$) encompasses the true risk. Formulae to determine these parameters are set out in Appendix C.







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Autosomal Reciprocal Translocations

Chapter: Autosomal Reciprocal Translocations
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RECIPROCAL TRANSLOCATIONS ARE COMMON, and every counselor can expect to see translocation families. The usual form is the simple, or two-way, reciprocal translocation: only two chromosomes, usually autosomes, are involved, with one breakpoint in each. It is this category we consider in this chapter. The special cases of translocations involving sex chromosomes, and of complex translocations, are dealt with in separate chapters.

The translocation heterozygote (carrier) may have a risk to have a child who would be mentally and physically abnormal due to a "segmental aneusomy." Typically, the imbalance is due to a segment of one of the participating chromosomes being duplicated and a segment of the other chromosome being deleted. This confers a partial trisomy and a concomitant partial monosomy. A few translocations are associated with a high risk, as much as 20%, or very rarely up to 50%, to have an abnormal child. Many translocations imply an intermediate level of risk, in the region of 5%–10%. Some carriers have a low risk, 1% or less; but the woman who is a carrier, or the partner of a male carrier, may have a high miscarriage rate. Others imply, apparently, no risk to have an abnormal child, but the likelihood of miscarriage is high. Yet others, discovered fortuitously, seem to be of no reproductive significance, with carriers having no difficulties in conceiving or carrying pregnancies and having normal children. The counselor needs to distinguish these different functional categories of translocation, in order to provide each family with tailor-made advice.

Biology

Simple reciprocal translocations arise when a two-way exchange of material takes place between two chromosomes. The process of formation follows the physical apposition of a segment of each chromosome, which may have been promoted by the presence in each segment of a similar DNA sequence. A break occurs in one arm of each chromosome, and the portions of chromosome material distal to the breakpoints switch positions. The distal portions exchanged are the *translocated segments*; the rest of the chromosome (which includes the centromere) is the *centric segment*. The rearranged chromosome is called a *derivative* (der) chromosome. It is identified according to which centromere it possesses, as in the der(5) and der(10) depicted in Figure **5–1**. When no loss or perturbation of genetic material occurs—in other words, the translocation is balanced—the phenotype of the heterozygote is normal, other things being equal. On classical cytogenetics, approximately 1 person in 500 is a reciprocal translocation heterozygote (Jacobs et al., 1992). The translocation may have arisen de novo in the person, or it may be widespread throughout a family, with many carriers, and sometimes of centuries-long duration. Koskinen et al. (1993) trace a t(12;21) in western Finland back to a couple born in 1752!

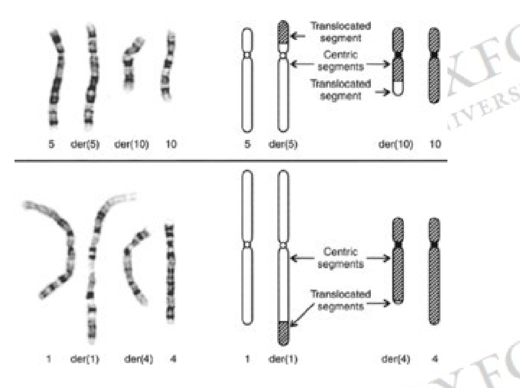


Figure 5–1
Reciprocal translocations demonstrating (*above*) double-segment and (*below*) single-segment exchange. The translocations are t(5;10)(p13;q23.3) and t(1;4)(q44;q31.3). (Cases of M. A. Leversha and N. A. Monk.)

When one of the translocated segments is very small and comprises only the telomeric cap of a chromosome arm—and thus we suppose contains no genes—this is regarded as being, effectively, a *single-segment* exchange. The t(1;4) translocation shown in Figure **5–1**, involving a substantial piece of chromosome 4 long arm exchanging positions with the terminal tip of a chromosome 1 long arm, exemplifies single-segment exchange. On the other hand, when both translocated segments are of substantial size, we refer to this as a *double-segment*[1] exchange. The translocation shown in Figure **5–1** between a chromosome 5 and a chromosome 10, with breakpoints in about the mid-short arm of

chromosome 5 and a little below the middle of the chromosome 10 long arm, is an example of a double-segment exchange. The translocation involving breakpoints right at, or actually within the centromere, with an exchange of entire arms, is a particular and rare type of double-segment exchange known as a *whole-arm* translocation (Vázquez-Cárdenas et al., 2007).

Details of Meiotic Behavior

At meiosis I in the primary gametocyte, the four chromosomes with segments in common come together as a foursome: a *quadrivalent*. To match homologous segments, the four chromosomes must form a cross-shaped configuration. This is most clearly seen when the chromosomes are at the pachytene stage (Fig. **5–2**). As meiosis progresses, the four components of the quadrivalent release their points of attachment except at the tips of the chromosome arms, and they form a ring; if attachment fails, or if one of the terminal pairings release, a chain forms instead of a ring (Oliver-Bonet et al., 2004). With breakdown of the nuclear envelope, spindles forming at each pole of the cell can track to the equator and seek attachment to the centromeres. A cellular motor comes into play, and the chromosome travels to one or other pole. According to which spindle attaches to which centromere—and this may in part be influenced by the configuration of the ring or chain—the distribution of the four homologs to the two daughter gametocytes is determined. Which homologs go to which pole is referred to as *segregation*. The expression *2:2 segregation* describes two chromosomes going to one daughter cell, and two to the other. In *3:1 segregation*, three chromosomes go to one daughter cell, and one to the other. In *4:0 segregation*, all four chromosomes go to one daughter cell, and none to the other.

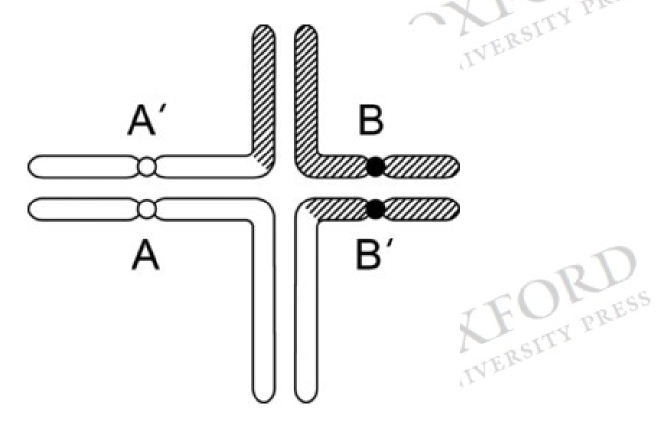




Figure 5–2
Pachytene configuration, simplified outline. The two normal (A, B) and the two translocation (A', B') homologs align corresponding segments of chromatin during meiosis I.

Modes of Segregation

Within these three broad categories, we can list the particular modes of segregation, according to which chromosomes actually go where. Referring to the four chromosomes of the quadrivalent as A, B, A′, and B′ (Fig. **5–2**), the modes of segregation are summarized as follows:

ONE DAUGHTER GAMETOCYTE WITH:	OTHER DAUGHTER GAMETOCYTE WITH:	SEGREGATION MODE
2:2 segregations		
A and B	A′ and B′	Alternate segregation
A and B′	B and A′	Adjacent-1 segregation
A and A′	B and B′	Adjacent-2 segregation
3:1 segregations		
A B A′	B′	3:1 segregation with
A B and B′	A′	tertiary trisomy or monosomy
A′ B′ and A	B	3:1 segregation with
A′ B′ and B	A	interchange trisomy or monosomy
4:0 segregation		
A B A′ B′	none	4:0 segregation with double trisomy or monosomy

Figure **5–3** depicts five of the possible pairs of daughter gametocytes. Other things being equal, the chromosomal combination is conserved through meiosis II, and the mature gamete forms. From one primary gametocyte, four spermatozoa, or one ovum and its polar bodies, are thus produced. Gametes from alternate segregation are normal or balanced. Conceptions from adjacent-1 gametes have trisomy for one translocated segment and monosomy for the other. Vice versa, adjacent-2 conceptions have trisomy for one centric segment and monosomy for the other. Tertiary aneuploidies have trisomy, or monosomy, with respect to the combined chromosomal content of one of the derivative chromosomes. Interchange aneuploidies have a full autosomal trisomy or a full monosomy. In 4:0 segregation, there is a double trisomy or a double monosomy. Some of the gametes with these unbalanced combinations may be "viable," in the sense of being "capable of giving rise to a conceptus, which would proceed through to the birth of a child." Mostly, in fact, they are not.



Figure 5–3
The categories of 2:2 and 3:1 segregation that may occur in gametogenesis in the translocation heterozygote. In the four 3:1 categories, only one of the two possible combinations in each category is depicted (both of each are shown in Fig. **5–4**).

> Recombination at meiosis I, and asymmetric segregation at meiosis II, can complicate the story, although this has little practical implication. If recombination occurs in the interstitial segment (between the centromere and the breakpoint), further unbalanced combinations are generated, most of which would not be remotely viable. This phenomenon may possibly have some relevance in preimplantation genetic diagnosis, since testing is done at a stage where there has been little opportunity for selective pressure to have applied. Scriven et al. (1998) list various of these recombination possibilities, and Van Hummelen et al. (1997) diagram the process with respect to a particular translocation on which they had undertaken sperm studies (and illustrate the point that a normal/balanced gamete can be restored following recombination in adjacent-1 segregation). The most telling evidence that recombination can happen comes from the observation of a meiosis I chromosome having one normal and one derivative chromatid, and polar body analysis has enabled such an observation to be made (Munné et al., 1998a). At meiosis II, asymmetric segregation may lead to two copies of a derivative chromosome being transmitted, as noted later in the section "Meiosis II Nondisjunction."

Alternate Segregation

In 2:2 alternate segregation, looking at each centromere in turn around the quadrivalent, one centromere goes to one pole, and the next centromere goes to the other pole. In other words, each centromere goes "alternately"[2] to one or the other pole. Thus, the two daughter cells come to contain, respectively, the two normal homologs in one, and the two derivative chromosomes in the other. Note that alternate segregation is essentially the only mode that leads to gametes with a complete genetic complement—one with a normal karyotype, the other with the reciprocal translocation in the balanced state. All other modes can be classified as *malsegregation*.

Adjacent Segregation

In 2:2 adjacent segregation, adjacent centromeres travel together ("adjacent" in the sense of centromeres being next to each other, in their positions around the quadrivalent). There are two categories. In *adjacent-1* segregation, adjacent chromosomes with unalike (nonhomologous) centromeres travel to the same daughter cell (an aide-mémoire: in adjacent-*1*, the daughter cells get *one* of each centromere). Overall, adjacent-1 is the most frequently seen mode of malsegregation in the children of translocation heterozygotes. In *adjacent-2* segregation, which is rather uncommon, adjacent chromosomes with like (homologous) centromeres go to the same daughter cell (another aide-mémoire: in adjacent-2, the *two* homologous centromeres go *to*gether). Thus, adjacent-2 segregation rather resembles nondisjunction.

3:1 Segregation

This is also referred to as 3:1 nondisjunction. Gametes with 24 chromosomes and 22 chromosomes are formed, and the conceptuses therefore have 47 or 45 chromosomes. Almost always, the 47-chromosome conceptus is the only viable one. Two categories exist: either the two normal chromosomes of the quadrivalent plus one of the translocation chromosomes go together to one daughter cell (*tertiary trisomy*) or, rarely, the two translocation chromosomes and one of the normal chromosomes segregate (*interchange trisomy*). Tertiary monosomy, with a 45-chromosome conceptus, is extremely rare. Interchange monosomy has never been seen, except at preimplantation genetic diagnosis (PGD).

4:0 Segregation

In autosomal translocations, 4:0 segregation has been regarded as being of academic interest only. But it may come to have some practical relevance in preimplantation genetic diagnosis.

In theory, 16 possible chromosomal combinations could be produced in the gametes of the autosomal translocation heterozygote. Four of these we can, for the most part, ignore (3:1 interchange monosomies and 4:0 segregants), because they are never viable. The two balanced gametes (2:2 alternate segregants) are always viable, other things being equal. Of the remaining 10 possibilities, it is common for none to be viable, with spontaneous abortion the universal outcome. If a translocation heterozygote does have the potential for viable imbalance in an offspring, it is most likely that there will be only one such combination (this was the case in 99% and 100% of translocations, in the considerable experience of two groups; Scriven et al., 1998). Usually, this sole survivable imbalance will be one that endows a partial trisomy. Infrequently two and, very rarely, more than two may be viable. Figure **5–4** depicts the various combinations that may be considered (using the previously discussed t(1;4) translocation as an example). In a review of 1159 translocation families, Cohen et al. (1994) found the proportions of chromosomally unbalanced offspring as follows: 71% adjacent-1, 4% adjacent-2, 22% tertiary trisomy/monosomy, and 2.5% interchange trisomy.

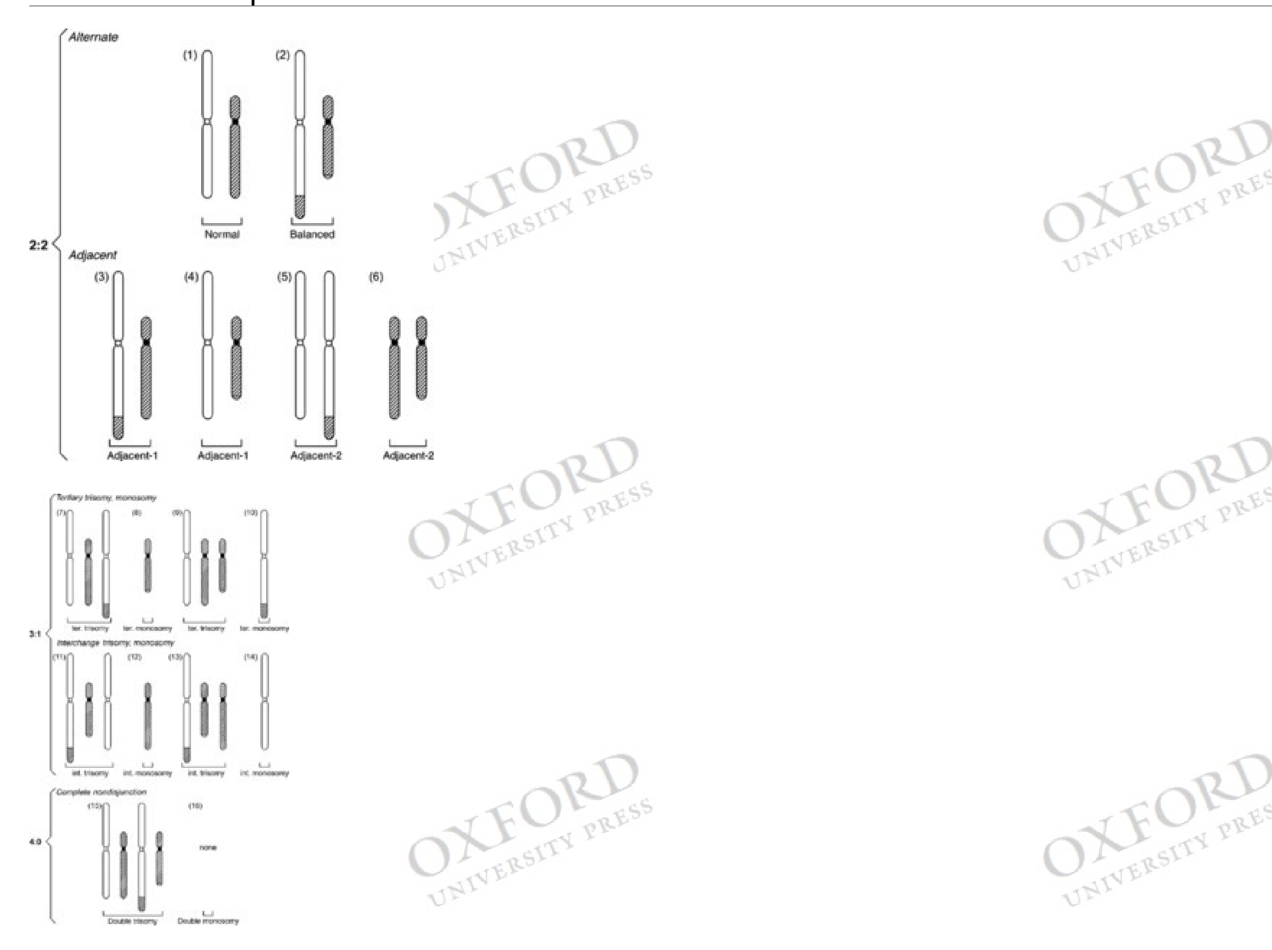


Figure 5–4
The full range of segregant gametes that may be produced by the translocation heterozygote, using the t(1;4) depicted in Figure **5–1** as an example. Chromosome 1 chromatin is shown open; chromosome 4 chromatin is crosshatched.

Gamete Studies.

It is, apparently, the norm for the heterozygote to produce gametes in which many of the possible chromosomal combinations occur, albeit the proportions may differ, and very substantially so, for some different translocations. Sperm karyotyping results from 44 men, heterozygous for a translocation, are summarized in Table 5–1, along with oöcyte karyotyping data (in most indirectly via polar body analysis) from seven women. Fifty-five percent of sperm, and 70% of ova, were chromosomally unbalanced. The great majority, if not all, of these studied individuals would have presented to the clinic because of reproductive difficulty, and so the data may possibly be biased in the direction of unbalanced forms, compared to the whole population of translocation heterozygotes. On average, alternate and adjacent-1 segregants are the predominant types in spermatogenesis, occurring in fairly similar fractions (43% and 32%, respectively). Adjacent-2 at 13% and 3:1 at 10% are less frequently seen; and barely any 4:0 segregant sperm. Considerable variation occurs: some heterozygotes had no 3:1 segregants, and one had 47%; for adjacent-2, the range is 0% to 40%. The spread of segregant types seems to be rather similar with men having the same translocation (Benet et al., 2005), such as the related individuals noted in Table 5–1. It would not be surprising if a fairly similar distribution and range of germ cell abnormalities were produced by their heterozygous sisters, although early data from preimplantation diagnosis research do suggest somewhat of a (possibly age-related) propensity in oögenesis for 3:1 segregation (Tables 5–1 and 5–2).

Table 5–1. Chromosome Segregations in Gametes of 51 Male and Female Reciprocal Translocation Heterozygotes, Shown as Percentages in Each Segregant Category

t	ALT	ADJ-1	ADJ-2	3:1	4:0
Male heterozygotes					
46,XY,t(1;2)(q32;q36)	41	42	6	11	0
46,XY,t(1;4)(p36.2;q31.3)[a]	46	38	7	9	0
46,XY,t(1;4)(p36.2;q31.3)[a]	39	50	8	3	0
46,XY,t(1;9)(q22;q31)	46	38	13	4	0
46,XY,t(1;11)(p36.3;q13.1)	33	43	16	8	0
46,XY,t(1;13)(q41;q42)	41	42	15	2	0
46,XY,t(2;3)(q24;p26)	55	36	7	1	0
46,XY,t(2;6)(p12;q24)	50	42	3	4	0

46,XY,t(2;9)(q21;p22)	43	28	24	4	0
46,XY,t(2;17)(q35;p13)	56	33	11	0	0
46,XY,t(2;18)(p21;q11.2)	42	35	14	8	0
46,XY,t(3 ;7)(q25;q36)	28	46	19	7	0
46,XY,t(3;8)(p13;p21)	34	44	21	1	0
46,XY,t(3;11)(q25.3;q25)	48	46	6	0.8	0
46,XY,t(3;15)(q26.2;q26.1)	48	36	12	2	2
46,XY,t(3;16)(p23;q24)	37	41	16	5	0
46,XY,t(3;19)(p21;p13.3)	39	36	22	3	0
46,XY,t(4;6)(q28;p23)	46	52	2	½	0
46,XY,t(4;8)(q28;p23)	35	33	20	11	0
46,XY,t(4;12)(p11;p11)	49	14	28	9	1
46,XY,t(4;17)(q21.3;q23.2)	57	35	7	2	0
46,XY,t(5;7)(q13;p15.1)	40	26	17	17	0
46,XY,t(5;11)(p13;q23.2)[b]	70	26	0	4	0
46,XY,t(7;14)(q11;q24.1)[b]	30	48	0	17	4
46,XY,t(5;13)(q11;q33)	77	21	2	0	0
46,XY,t(5;18)(p15;q21)	81	16	0	3	0
46,XY,t(6;9)(p12;q13)	24	14	40	22	0
46,XY,t(7;8)(q11.21;cen)[a]	57	25	11	7	0.04
46,XY,t(7;8)(q11.21;cen)[a]	63	18	13	7	0.3
46,XY,t(8;9)(q24.2;q32)	44	41	3	9	0.6
46,XY,t(8;22)(q24.22;q11.21)	24	15	19	42	0
46,XY,t(9;10)(q11;p11.1)	56	13	9	21	0
46,XY,t(9;22)(q21;q11.2)	56	26	11	6	0.6
46,XY,t(10;14)(q24;q32)	45	39	12	5	0
46,XY,t(11;17)(q13.1;p11.2)	41	26	26	7	0
46,XY,t(11;22)(q23;q11)	22	14	32	30	0
46,XY,t(11;22)(q23;q11)	27	18	13	40	0.5
46,XY,t(11;22)(q25;q12)	29	22	15	35	0
46,XY,t(14;20)(p11.2;p11.1)	51	19	21	4	0
46,XY,t(15;17)(q21;q25)[a]	51	35	9	3	0
46,XY,t(15;17)(q21;q25)[a]	49	38	8	3	0
46,XY,t(15;17)(q21;q25)[a]	48	40	9	2	0
46,XY,t(15;17)(q21;q25)[a]	53	34	11	1.5	9
46,XY,t(15;22)(q22;q13)	19	16	16	43	0

46,XY,t(17;22)(q11;q12)	19	13	6	47	0
Average fractions* (sperm)	43%	32%	13%	10%	~0
	Total abnormal = 55%				
Female heterozygotes					
46,XX,t(2;14)(q23;q24)	11	22	22	44	0
46,XX,t(2;14)(q31;q24)	14	57	14	14	0
46,XX,t(4;14)(p15.3;q24)	27	55	0	18	0
4 6,XX,t(6;21)(q13;q22.3)	0	50	0	50	0
46,XX,t(7;20)(q22;q11.2)	50	17	0	33	0
46,XX,t(9;11)(p24;q12)	100	0	0	0	0
46,XX,t(14;18)(q22;q11)	40	0	60	0	0
Average fractions** (ova)	30%	30%	14%	26%	0
	Total abnormal = 70%				

Notes: The sperm data are arbitrarily set out according to the methodology used. Those down to the t(5;18) were analyzed using the human-hamster hybrid model; the remainder, from the t(6;9), were based upon fluorescence in situ hybridization (FISH) analysis of interphase sperm nuclei (the results from the two approaches are quite similar). The t(7;14) is "out of order"; it is placed beneath the t(5;11) also carried by this subject.[b] The full dataset according to the two methodologies is contained in Benet et al. (2005), with a total of 89 individuals analyzed.

Roundings of percentages have resulted in some data not quite totaling 100%. Some sperm FISH cases were interpreted as showing "other" combinations; these are not listed, and the totals here come to less than 100%.

* Sperm: average of the percentages in Tables 1 and 3 in Benet et al. (2005).

** Ova: average of pooled oöcyte observations.

[a] Related individuals; note the quite similar fractions.

[b] Both translocations carried by the same man, as a double heterozygote; note the very different proportions from each translocation.

Alt, alternate; adj-1, adjacent-1; adj-2, adjacent-2.

Sources: Sperm data from Benet et al. (2005). Oöcyte/polar body data, which naturally are based on much smaller numbers (2–11 observations per woman), from Munné et al. (1998b, 1998c), Conn et al. (1999), and Escudero et al. (2000).

Table 5–2. Chromosome Segregations in Embryos of 16 Reciprocal Translocation Heterozygotes Studied at Preimplantation Diagnosis (Shown as Actual Numbers in Each Segregant Category)

t	ALT	ADJ-1	ADJ-2	3:1	4:0
Male Heterozygotes					
46,XY,t(3;6)(q25;q23)	3	7	2	1	0
46,XY,t(3;7)(q23;q36)	3	1	3	0	0
46,XY,t(3;7)(q25.3;p22.1)	4	1	3	1	0
46,XY,t(11;22)(q23.3;q11.2)	9	4	1	0	1
Average fractions	43%	30%	20%	5%	2%
	Total abnormal = 57%				
Female Heterozygotes					
46,XX,t(1;13)(q23;p11)	0	1	0	1	0
46,XX,t(1;19)(q32.1;q13.1)	11	1	3	6	0
46,XX,t(2;4)(p22.2;q33)	2	5	0	0	0
46,XX,t(3;5)(p12;q14.2)	2	1	0	0	0
46,XX,t(4;15)(q26;q13)	0	0	0	1	0
46,XX,t(5;14)(p15.1;q32.1)	4	2	0	3	0
46,XX,t(8;18)(p21.1;q21.1)	2	0	0	0	0
46,XX,t(9;20)(q34.2;q11.2)	5	3	0	0	0
46,XX,t(11;17)(p15.5;p13)	9	7	0	5	1
46,XX,t(11;22)(q23.3;q11.2)	0	0	0	2	1
46,XX,t(12;17)(p13;p13)	11	4	0	2	0
46,XX,t(14;22)(q11.2;q13.3)	6	0	3	1	0
Average fractions	50%	23%	6%	20%	2%
	Total abnormal = 50%				

Notes: See also Table 25–2. Note that more recent data from ESHRE indicates a higher abnormality rate (about 80%) than the 50%–57% shown here (see text).

Alt, alternate; adj-1, adjacent-1; adj-2, adjacent-2. Average fractions are derived from pooling the data in each group.

Source: From Mackie Ogilvie and Scriven (2002).

Conceptions.

It might be expected that the distribution of normal and abnormal conceptions would reflect the distributions of karyotypes in the gametes. Thus, the two men in Table 5–1 with more than 60% alternate segregant forms, they being heterozygous for a t(6;14) and a t(10;12), respectively, might logically be presumed to have a better chance of having a normal child than, for example, the man with a t(1;11) in whose sperm only 33% showed a normal or balanced karyotype. This may be true in some cases, but not necessarily so in all instances, as research from the PGD laboratory is now revealing.

Coonen et al. (2000) reported a man with a t(3;11)(q27.3;q24.3), who had 45% alternate forms of segregation on sperm analysis, but of 18 biopsied day-3 cleavage embryos, only three (17%) were normal or balanced. (One of these tested embryos was implanted, and a normal daughter with the balanced translocation subsequently born.) Thus, the fraction of normal/balanced karyotypes fell by one third, from the gametes to the conceptions. His case, as it has turned out, may not be atypical. Initially, in comparing the pooled segregant distributions of gametes and embryos in each sex, it had appeared that the numbers overall were not grossly discrepant (Table 5–2). However, more recent data published from the ESHRE PGD Consortium have shown that about 80% of embryos from translocation carrier couples are chromosomally abnormal (Goossens et al., 2008). Of 7871 successfully diagnosed embryos, only 1597 (20%) were “transferable,” the fractions nearly identical from male or female carriers. This fraction of 80% is well above the 55% proportion of abnormal sperm (Table 5–1). Very similar data comes from Lim et al. (2008b) and Ko et al. (2010), with ~20% of PGD embryos being normal or balanced; these two groups observed all patterns of malsegregation, including 4:0.

Acrocentric chromosomes participating in a reciprocal translocation might be expected to influence segregation, due to the very small lengths of their short arms and thus a marked asymmetry of the quadrivalent (Benet et al., 2005). Lim et al. (2008b) were able to demonstrate the truth of this proposition, in their PGD study mentioned earlier. Those translocations that involved an acrocentric chromosome had fewer alternate segregants compared to those that did not (15% cf. 26%), but more 3:1 malsegregants (27%

cf. 20%) in 508 embryos analyzed.

Viability In Utero.

Most unbalanced combinations would produce such enormous genetic imbalance that the conceptus would be lost very early in pregnancy (occult abortion), or even fail to implant. Moderate imbalances would proceed to the stage of recognizable miscarriage or to later fetal death in utero. Only those conceptuses with lesser imbalances might result in the birth of an abnormal child.

Viability is much more likely in the case of effective single-segment imbalance, with only one segment of substantial size. In the unbalanced state, a partial monosomy or trisomy for the other very small terminal segment is likely to contribute minimally or (if it contains no genes, or at any rate no "dosage vulnerable" genes) not at all to the overall imbalance. This is of particular relevance in adjacent-1 segregation. Consider, for example, gamete (3) in Figure **5–4**. The material missing from the telomeric tip of chromosome1 long arm—the telomeric cap—is so small that its loss is, as far as we can tell, of insignificant phenotypic effect. For practical purposes, we can ignore this partial monosomy. So the significant imbalance reduces to a partial 4q trisomy (trisomy 4q31.3-qter). This, as it happens, is well recognized as being a viable complement (and it is the imbalance in the children whose photograph appears in the frontispiece). On the other hand, in the double-segment exchange the imbalance contributed by each segment must be taken into account. Thus, adjacent-1 gametes have both a partial trisomy and a partial monosomy to a significant degree and would produce a "phenotypic hybrid." Very frequently, the combination is nonviable.

> If very early miscarriages could be karyotyped, one might expect to discover more of the imbalanced forms. Fritz et al. (2000) conducted such an exercise, using comparative genomic hybridization as the cytogenetic tool. They had identified a family segregating a subtle t(4;12)(q34;p13), in which two children had been born with 46,der(4),t(4;12)(q34;p13), giving a distal 4q monosomy. There had been five previous abortions, and archival pathology material (paraffin-embedded placental tissue) was available for analysis from three of these. A 12- and a 17-week abortus both showed the same karyotype as the surviving children. An 8-week abortus, described as a hydatidiform mole, karyotyped as a tertiary trisomy for almost the whole of chromosome 4: 47,XY,+der(4),t(4;12)(q34;p13), combination (9) in Figure **5–4**.

Predicting Segregant Outcomes

How can we determine, for the individual translocation carrier, which segregant outcomes, if any, might lead to the birth of an abnormal child? What might be the relative roles of an inherent tendency for a particular type of segregation to occur, and of in utero selection against unbalanced forms? A useful approach is to imagine how the chromosomes come to be distributed during meiosis. Following Jalbert et al. (1980, 1988), we may draw, roughly to scale, a diagram of the presumed pachytene configuration of the quadrivalent, and then deduce which modes of segregation are likely to lead to the formation of gametes, which could then produce a viable conceptus. The following, and with reference to Figure **5–5**, are the ground rules:

1. We assume that alternate segregation is *(a)* frequent and *(b)* associated with phenotypic normality.
2. The least imbalanced, least monosomic of the imbalanced gametes is the one most likely to produce a viable conceptus.
3. If the translocated segments are small in genetic content, adjacent-1 is the most likely type of malsegregation to be capable of giving rise to viable abnormal offspring (Fig. **5–5a**).
4. If the centric segments are small in content, adjacent-2 is the most likely segregation to give a viable abnormal outcome (Fig. **5–5b**).
5. If one of the whole chromosomes of the quadrivalent is small in content, 3:1 disjunction is the most likely (Fig. **5–5c**). The small chromosome may be a small derivative chromosome or a chromosome 13, 18, or 21.
6. If the quadrivalent has characteristics of both Rules 3 and 5, or of Rules 4 and 5, then both adjacent and 3:1 segregations may give rise to viable offspring.
7. If the translocated and centric segments both have large content, no mode of segregation could produce an unbalanced gamete that would lead to a viable offspring (Fig. **5–5d**).
8. Subtelomeric translocations may not necessarily form a quadrivalent, and the pairs of homologs might simply join up as bivalents, each pair then segregating independently.

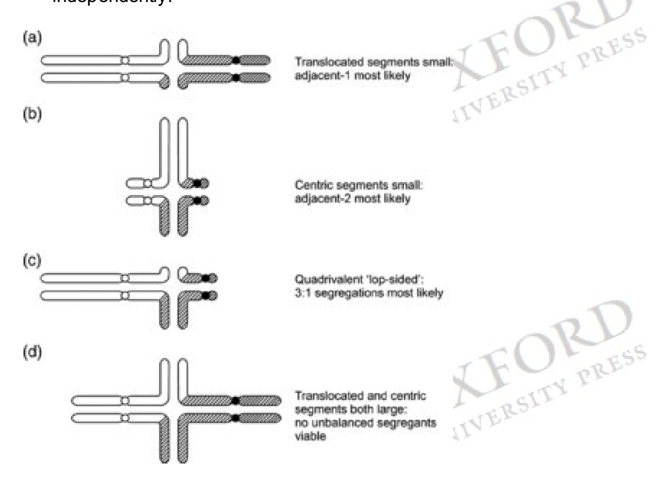




Figure 5–5
Prediction of likely viable segregant outcomes by pachytene diagram drawing and assessment of the configuration of the quadrivalent.

Some examples to illustrate these points follow.[3]

Adjacent-1 Segregation, Single-Segment Exchange

Many translocations involve an effectively single-segment exchange, with the "single" translocated segment comprising a fairly small amount of chromatin (1%–2% of the haploid autosomal length, or HAL). This is the classical scenario for adjacent-1 segregation to occur, and to produce a phenotype capable of postnatal survival. The family with the t(1;4) in Figure **5–1**, whose children with partial 4q trisomy are shown in the frontispiece as discussed earlier, provides an example.

Consider now the family whose pedigree is depicted in Figure **5–6a**, in which the individuals shown as heterozygotes have the balanced translocation 46,t(3;11)(p26;q21). A segment of chromatin consisting of almost half of the long arm of chromosome 11, and comprising 1.4% of the HAL, is translocated to the tip of chromosome 3 short arm (Fig. **5–6b**). The telomeric tip of chromosome 3 short arm, which we imagine to comprise little or no phenotypically important genetic material, has moved reciprocally across to chromosome 11. The presumed pachytene configuration during gametogenesis in the heterozygote would be as drawn in Figure **5–6c**. The adjacent-1 segregant gamete with der(3) plus normal 11 (heavy arrows) produces a conceptus that has a partial 11q trisomy, since the der(3) carries the segment 11q21-qter. The loss of the 3p telomeric tip in this der(3) we imagine to have no effect. Two, probably three children in the family had been born with this karyotype. No individuals are known having the other adjacent-1

combination (Fig. **5–6c**, light arrows), that is to say, the 46,+der(11) karyotype, which would endow a partial 11q monosomy. Consulting Schinzel (2001), viability for the segment 11q21-qter in monosomic state is recorded in only two cases. We assume, therefore, that it has a very high lethality in utero.

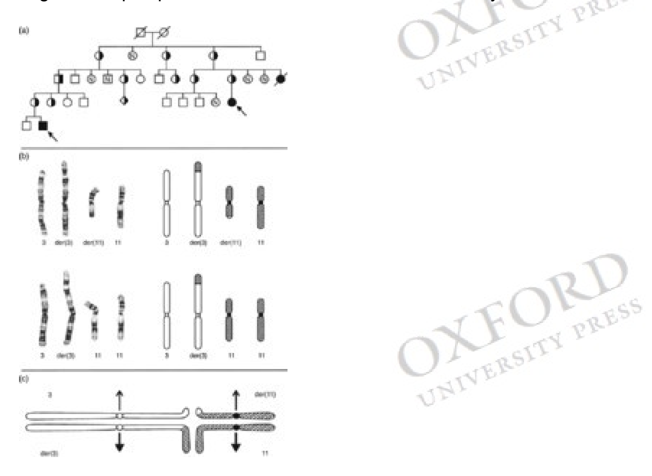




Figure 5–6
Adjacent-1 segregation. (*a*) Pedigree of a family in which there segregates a t(3;11)(p26;q21) having the characteristics associated with adjacent-1 malsegregation. Two independently ascertained probands have a partial 11q trisomy, and a deceased relative, who died at age 18 in an institution for the retarded, had a similar appearance from photographs, and so very probably had the same karyotype. Filled symbol, unbalanced karyotype; half-filled symbol, balanced carrier; N in symbol = 46,N; small diamond, prenatal diagnosis; arrow, proband. (*b*) Partial karyotype of a translocation heterozygote (*above*), showing the 3;11 translocation, and a child with the unbalanced complement (*below*) (case of A. J. Watt). (*c*) The presumed pachytene configuration during gametogenesis in the heterozygote (chromosome 3 chromatin, open; chromosome 11 chromatin, crosshatched). Arrows indicate movements of chromosomes to daughter cells in adjacent-1 segregation; heavy arrows show the combination observed in this family.

The scenario of a single survivable imbalanced form, due to a partial trisomy from adjacent-1 segregation in a “single-segment” translocation, as in this t(3;11) example, is, as mentioned earlier, much the most commonly encountered circumstance in translocation families at risk for an abnormal child.

Infrequently, both the partial trisomic and the partial monosomic forms are observed. A good example of this is given by distal 4p translocations: both deletion and duplication for this segment are well recognized as having substantial in utero viability. Consider the translocation t(4;12)(p14;p13) described in a family study in Mortimer et al. (1980). A number of family members over three or more generations were balanced carriers, and abnormal children had been born with typical Wolf-Hirschhorn syndrome (all dying in infancy), while others presented the syndrome of partial 4p trisomy (all surviving at least well into childhood). The breakpoints of the translocation are in distal 4p and at the very tip of 12p (12pter). The presumed pachytene configuration would be as drawn in Figure **5–5a** (imagining the chromosome 4 chromatin open and chromosome 12 chromatin crosshatched). With such short translocated segments (and very long centric segments), adjacent-1 segregation is the only possibility for viable imbalance. If we ignore the tiny contribution of a duplication or deletion for telomeric 12p—in other words, if we interpret this as an effective single-segment imbalance—the situation reduces to the two possible adjacent-1 outcomes being a partial 4p trisomy and a partial 4p monosomy. Both of these are recognized entities, as noted earlier, and apparently both have substantial viability in utero. The abnormal karyotypes would be written 46,der(12)t(4;12)(p14;p13) and 46,der(4)t(4;12)(p14;p13).

If the “other segment” comprises acrocentric short arm, then, given the redundancy of this material, the case for considering the translocation as a single-segment entity is particularly valid, with the resulting imbalances being “pure.” De Carvalho et al. (2008) illustrate this in their report of a large family segregating a t(5;15)(p13.3;p12) translocation, in which the extraordinary total of 21 individuals were known with either a deletion or a duplication of distal 5p. Applying the principles set out later in this chapter, the risk to the carrier in this family to have a child with an imbalance—presumably due to adjacent-1 malsegregation—is very high, at 54%. Essentially, as it would seem, alternate and adjacent-1 segregant outcomes were equally probable.

Adjacent-1 Segregation, Double-Segment Exchange

With a double-segment translocation, an adjacent-1 imbalanced conceptus has both a partial trisomy and a partial monosomy (also called a duplication/deficiency, or duplication/deletion, abbreviated to dup/del). The combined effect of the two imbalances is more severe than either separately. Thus, it is infrequent that the carrier of a “double-segment” exchange can ever have a chromosomally unbalanced pregnancy proceeding through to term, or close to term. Multiple miscarriage is the typical observation (e. g., Fig. **5–16**). But occasionally viability is observed for one, or rarely both, of the dup/del combinations. Nucaro et al. (2008) studied a t(3;10)(p26;p12) family with affected individuals in three generations, and yet all still living, and able to be examined and their karyotypes confirmed as 46,der(3)t(3;10)(p26;p12), conveying a partial 3p monosomy and 10p trisomy; the countertype adjacent-1 karyotype was not observed, but it may well have been the cause of the several miscarriages recorded. The double-segment t(4;8)(p16.1;p23.1) depicted in Figure **5–7** has very small translocated segments: the tip of chromosome 4 and the tip of chromosome 8 have exchanged positions.[4] In this family, each of the two possible adjacent-1 segregant outcomes were observed: the index case with del(4p)/dup(8p), and his uncle with dup(4p)/del(8p). In the former, a Wolf-Hirschhorn gestalt was discernible, reflecting the del(4p) component. A similar example is seen in the family reported in Rogers et al. (1997). They provide in their paper a photograph of six siblings sitting on a sofa in 1958, one with a dup(11q)/del(4q) karyotype, two who since died presumed to have been del(11q)/(dup(4q), and one girl carrying the family t(4;11)(q34.3;q23.1) who went on to have, in the next generation, a del(11q)/(dup(4q) child.

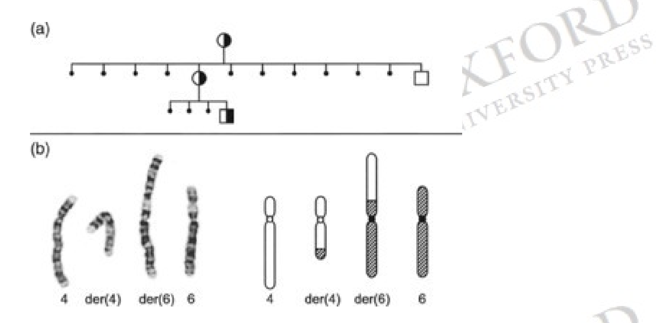


Figure 5–16
No unbalanced product viable. (*a*) Pedigree of a kindred in which mother and daughter have had multiple miscarriages, each having (*b*) the karyotype 46,XX,t(4;6)(q25;p23). (Case of A. J. Watt.) The presumed pachytene configuration during gametogenesis in the heterozygote would be as in Figure **5–5d** (chromosome 4 chromatin, open; chromosome 6 chromatin, crosshatched) and, with large centric and translocated segments, the translocation has none of the features that enable viability of any unbalanced

segregant combination.

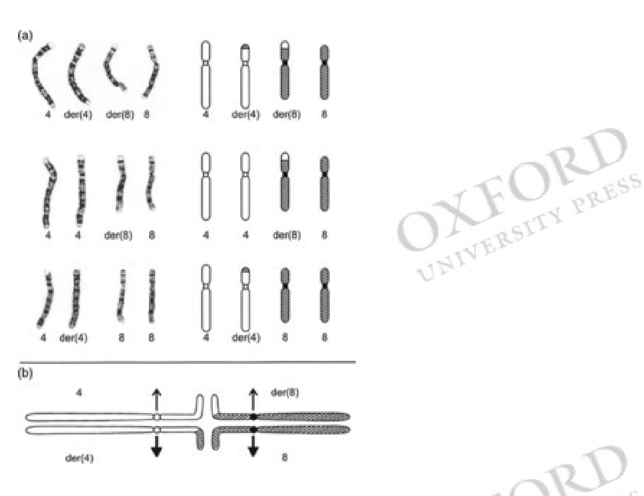




Figure 5–7
Adjacent-1 segregation, double-segment translocation with very small segments. (*a*) Parent with the translocation t(4;8)(p16.1;p23.1). The index case, his child, has the karyotype 46,+der(4) and so has a del(4p)/dup(8p) imbalance, and an uncle has the countertype dup(4p)/del(8p) imbalance due to the 46,+der(8) karyotype. (Case of C. E. Vaux.) (*b*) The presumed pachytene configuration during gametogenesis in the heterozygote (chromosome 4 chromatin, open; chromosome 8 chromatin, crosshatched). Arrows indicate movements of chromosomes to daughter cells in adjacent-1 segregation. The upper combination (light arrows) would produce the dup(4p)/del(8p) imbalance, and the lower (heavy arrows) the del(4p)/dup(8p) imbalance.

Exceptionally, both translocated segments can be of substantial size and yet be survivable, if barely, to term. The outlying points in Figure **5–17** reflect such cases. The double-segment t(5;10)(p13;q23.3) exchange illustrated in Figure **5–1** provides an example, this translocation having been identified in a family following the death of a neonate with multiple malformations. The genetic abnormality comprises a deletion of 5p and a duplication of 10q, for a total imbalance of 2.5% HAL (1.1% HAL monosomy plus 1.4% HAL trisomy).

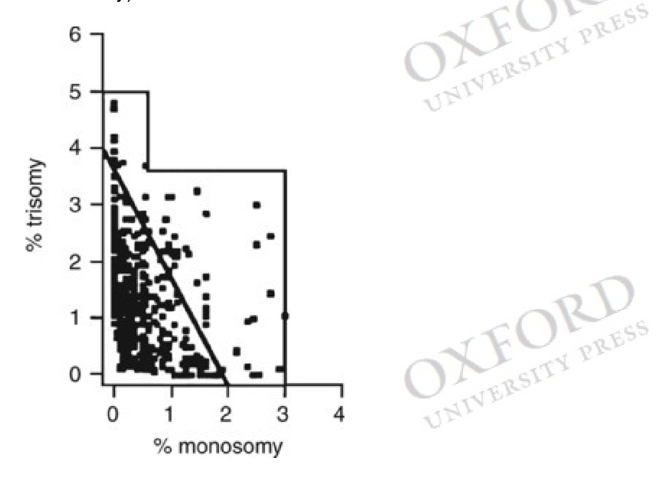




Figure 5–17
Viability of combined duplication/deletion states, according to amount of imbalance, measured as % HAL. Most (96%) fall within the triangular area whose hypotenuse lies between 4% duplication/0% deletion and 2% deletion/0% duplication, and a few outliers define an envelope of viable imbalances. (From O. Cohen et al. 1994, Viability thresholds for partial trisomies and monosomies. A study of 1,159 viable unbalanced reciprocal translocations, *Human Genetics* 93:188–194. Courtesy O. Cohen, and with the permission of Springer-Verlag.)

When entire arms of chromosomes are translocated (whole-arm translocation), it is almost always so that the unbalanced segregants would be unviable (Vázquez-Cárdenas et al., 2007). Rare exceptions exist. Czakó et al. (2002) report a t(18;20)(p11.1;p11.1), in which the abnormal child of a carrier father was effectively trisomic for all of 20p and monosomic for all of 18p (1.0% HAL trisomy plus 0.8% HAL monosomy). The woman with a whole-arm 15p;16q translocation described in Chen et al. (2004d) had a history of miscarriage and stillbirth, and two further pregnancy losses proven to have complete 16q trisomy, this imbalance conveying as much as 2.1% HAL trisomy, and associated with a recognized severe phenotype. (Since the concomitant 15p monosomy presumably did not contribute to the fetal defects, this example has more of the flavor of a single-segment translocation.)

The opportunity occasionally arises to provide direct evidence of early in utero lethality of a particular imbalanced state. In a family study of a t(8;18)(p21.3;p11.23), Cockwell et al. (1996b) demonstrated on a severely malformed spontaneously aborted 11-week fetus one of the adjacent-1 conceptions, the dup(8p)/del(18p) state. This chromosomal constitution caused a double-segment imbalance, with a trisomy for 8p21.3-pter, and a monosomy for 18p11.23-pter, giving a combined 1.2% HAL imbalance (0.8% for trisomy, 0.4% for monosomy). The countertype dup(18p)/del(8p) karyotype had produced, in this family, a child with an abnormal phenotype. Atypically, this viable form had more HAL monosomy than trisomy.

Adjacent-1 Segregation with Subtelomeric Double-Segment Exchange

With subtelomeric fluorescence in situ hybridization (FISH) or microarray, translocations involving submicroscopic (typically <2–3 Mb) segments in the (generally) gene-rich regions just below the telomeres can be identified. Consider the family reported in Vogels et al. (2000), the pedigree of which is presented in Figure **5–8a**. Three individuals (two cousins and the nephew of one of them) presented a similar picture of severe mental defect and “obnoxious” behavior, with facial dysmorphism and certain malformations; another with similar facial dysmorphism had died of a heart defect as a neonate. This picture demanded close attention to the possibility of a chromosomal explanation, but

high-resolution karyotyping was normal in all four, with one of the cousins having been tested on several occasions. Finally, FISH with subtelomeric probes provided a clear and unambiguous illumination (Fig. **5–8b**). Testing the potential carriers in the family, a 5q subtelomeric probe and an 18q subtelomeric probe hybridized properly to one chromosome 5qter and to one chromosome 18qter; but the other 5qter hybridized with the 18q probe, and the other 18qter carried the 5q probe. Thus was demonstrated a double-segment reciprocal exchange between 5q and 18q: 46,t(5;18)(subqter;subqter).[5] The affected individuals were trisomic for a very small segment of subterminal 5q, and monosomic for a similarly small segment of 18qter, and the clinical picture suggested features of both the dup(5q) and the del(18q) syndromes.

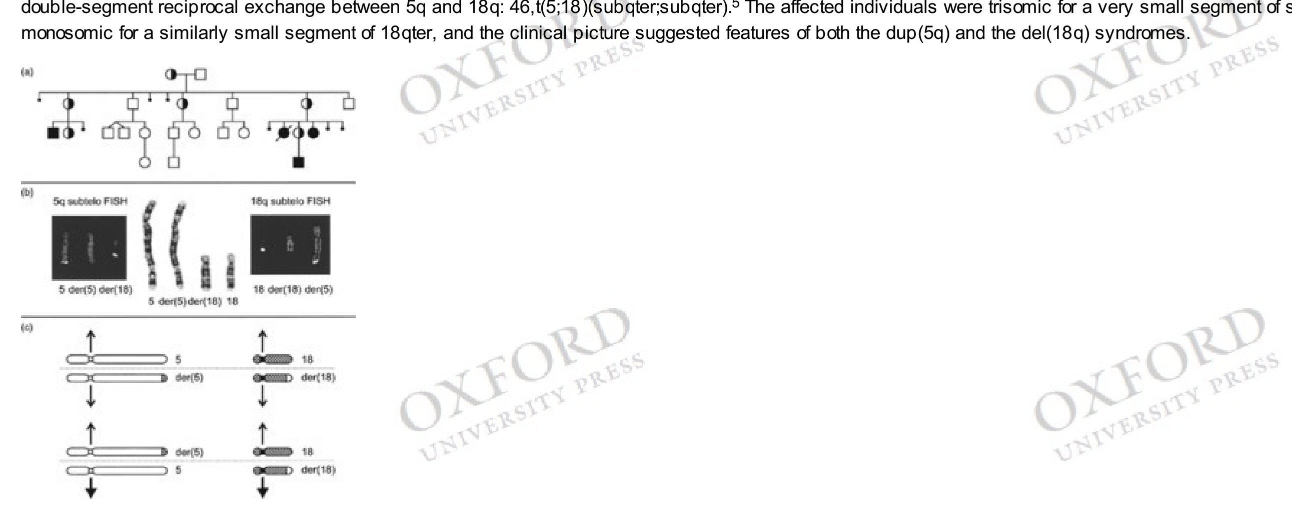


Figure 5–8

The subtelomeric translocation. (*a*) Pedigree of family. Filled symbol, abnormal individual with subtelomeric aneusomy; half-filled symbol, balanced carrier; dot, miscarriage. (From Vogels et al., 2000; courtesy J.- P. Fryns.) (*b*) Fluorescence in situ hybridization (FISH) demonstration of a reciprocal translocation t(5;18), undetectable on high-resolution G-banding (*middle*), but obvious using probes for the 5q subtelomeric region (*left*), and the 18q subtelomeric region (*right*). (*c*) Segregation patterns at meiosis I in the heterozygote, making the assumption, which may or may not be correct, that the homologs align as independent bivalents, rather than as a quadrivalent. Segregation would thus be 1:1 for each pair of bivalents. If the pairs of bivalents happen to be disposed on either side of the equatorial plate as in (*c*) *upper*, normal and balanced daughter cells are produced. If they happen to be disposed as in (*c*) *lower*, unbalanced cells result, either with del(5q)/dup(18q) (thin arrows), or, as observed in this family, dup(5q)/del(18q) (thick arrows). The equatorial plate, in transverse section, is indicated by the dotted line. This scenario will commonly apply to the submicroscopic reciprocal translocation identified following microarray analysis on an index case.

A number of similar families are now being reported, and many genetics services are reviewing their "chromosomal-seeming families" in which classical cytogenetics had failed to reveal any abnormality. A recurring theme in microarray analysis is the recognition of a terminal deletion accompanied by duplication of terminal material from another chromosome (e.g., Izumi et al., 2010); and when probes for the specific regions are "FISHed back" to a metaphase spread, the duplicated segment can be seen as translocated to the deletion site. Many will be de novo, but a proportion are the consequence of a balanced parental reciprocal translocation.

If a quadrivalent were to form in a subtelomeric translocation, practically always it would only ever be the adjacent-1 gametes that would be viable, besides the normal and balanced forms. Furthermore, it is unlikely that any other segregation pattern than 2:2 would occur. With such tiny chromosomal segments involved, it is probable that the homologs, the normal and the derivative, would simply pair up as in a normal bivalent, leaving the tiny nonhomologous segments at the ends unpaired. In that case, the expected segregations at meiosis would be random, with equal probability for each outcome, namely, normal:balanced:(dup/del):(del/dup) in the ratio 1:1:1:1.

Adjacent-2 Segregation

This is an uncommonly observed mode of segregation, typically limited to translocations in which the two participating chromosomes each has a short arm of small genetic content, and small enough that the whole short arm can be viable in the trisomic state. In fact, most cases involve an exchange between chromosome 9 and an acrocentric, or between two acrocentrics (Duckett and Roberts, 1981; Stene and Stengel-Rutkowski, 1988; Chen et al., 2005d). The breakpoints characteristically occur in the upper long arm of one chromosome and immediately below the centromere in the long arm of the other (an acrocentric). Thus, the centric segments are small.

The t(9;21)(q12;q11) illustrated in Figure **5–9a** exemplifies the adjacent-2 scenario. At meiosis I, the form of the quadrivalent would be as drawn in Figure **5–9b**. The "least imbalanced, least monosomic" gamete from 2:2 malsegregation is the one receiving chromosome 9 and the der(9) (heavy arrows). The conceptus will have, in consequence, a duplication of 9p (and a small amount of 9q heterochromatin) and a deletion of 21p (and a minuscule amount of subcentromeric 21q). Although comprising a substantial piece of chromatin (1.8% of HAL), 9p is qualitatively "small" in the trisomic state. Monosomy for 21p is without effect, and the 21q loss makes little if any contribution, and thus the picture is practically that of a pure 9p trisomy. This is a known viable aneuploidy. The countertype gamete with the der(21) causes monosomy 9p and is not viable. A very similar circumstance applies with the t(4;13)(q12;q12) described in Velagaleti et al (2001); the open and crosshatched chromosomes in the cartoon karyotype (Fig. **5–9**) could be regarded, for this example, as chromosomes 4 and 13, respectively. The index case in this family was trisomic for all of 4p, and the small segment 4cen-q12 (and monosomic for the tiny segment 13p-q12), having the karyotype 46,XY,+der(4),–13.

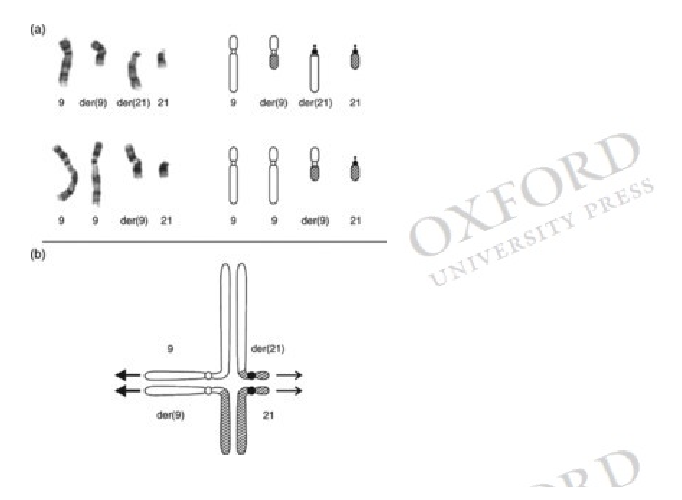




Figure 5–9
Adjacent-2 segregation. (*a*) Mother (*above*) has a reciprocal translocation t(9;21)(q12;q11), and her child (*below*) has the adjacent-2 karyotype 46,+der(9)t(9;21)(q12;q11). (Case of C. M. Morris and P. H. Fitzgerald). (*b*) The presumed pachytene configuration during gametogenesis in the heterozygote (chromosome 9 chromatin, open; chromosome 21 chromatin, crosshatched). Arrows indicate movements of chromosomes to daughter cells in adjacent-2 segregation; heavy arrows show the viable combination, as observed in this family.

The del(22)(q11) syndrome, so well known otherwise due to microdeletion, can also arise from a familial translocation, and this provides an example of a double-segment imbalance with adjacent-2 segregation. Imagine a t(9;22)(q12;q11.21) with the 22q breakpoint just below the DiGeorge critical region (DGCR). Considering the crosshatched chromosome in Figure **5–9b** to be a chromosome 22, then the der(9) will lack the DGCR. A 46,+der(9),–22 child from adjacent-2 segregation (the heavy arrows) will have the 22q deletion syndrome, superadded upon a 9p trisomy. Pivnick et al. (1990) and El-Fouly et al. (1991) describe children in whom these separate-and-together dup(9p) and del(22q) phenotypes could be distinguished.

A double-segment exchange with both adjacent-2 segregants observed, and reflecting a parent-of-origin effect, is shown in the family reported by Abeliovich et al. (1996). The family translocation, carried by the father, was due to breakpoints in the long arms of chromosomes 15 and 21, t(15;21)(q15;q22.1). Both centric segments, 15pter-15q15 and 21pter-21q22.1, are of quite substantial size. One child had the karyotype 46,–15,+der(21), with a proximal partial 15q monosomy and a proximal partial 21q trisomy. The phenotype was predominantly that of the Prader-Willi syndrome (PWS), reflecting the lack of a *paternally* contributed PWS critical region, residing in 15q11-q13. There was no clearly apparent contribution from the partial trisomy for 21pter-21q22.1. The other child, with a dup(15q)/del(21q) combination, 46,+der(15),–21, displayed a combination of features due to monosomy 21pter-21q22.1 and trisomy 15pter-15q15. An analogous story is that of a mother carrying a translocation t(15;22)(q13;q11.2), and in this case her child with the 46,–15,+der(22) combination presented the clinical picture of Angelman syndrome (AS), due to absence of a *maternally* originating AS critical region in 15q11–13 (Kosaki et al., 2009). Another child of hers had the opposite adjacent-2 imbalance, 46,–22,+der(15), and his phenotype was that of DiGeorge syndrome.

A double-segment case in which the two centric segments were much smaller is exemplified in Chen et al. (2005d). Here, in a 14;21 rearrangement, described as t(14;21)(q11.2;q11.2), both breakpoints were in the first subband below the centromere. The der(14) thus comprised almost all chromosome 21 material, with just the short arm, centromere, and a very small amount of proximal long arm being from chromosome 14; and vice versa, the der(21) consisted largely of chromosome 14 material. Three affected family members, two brothers and their aunt, carried the der(14) in unbalanced state due to adjacent-2 segregation and were thus trisomic for the small proximal 14q segment and monosomic for the small proximal 21q segment. The dysmorphology was quite mild, but the functional neurobehavioral phenotype was rather severe. A very similar (and possibly identical) scenario is described in Dave et al. (2009): the carriers in this family typed 46, t(14;21)(q21.2;q21.2), and the three affected individuals as 46,XX,+der(14)t(14;21)(q21.2;q21.2),–21. This sort of translocation, with very proximal q arm breakpoints in acrocentric chromosomes, may require microarray, with FISH confirmation, for its recognition, as Koochek et al. (2006) show in a t(14;15)(q11.2.;q13.3). Affected individuals inheriting a duplication of proximal 15q due to a maternal adjacent-2 malsegregation displayed a phenotype of which autism was a prominent feature.

The reason so few examples of adjacent-2 segregants are seen is that most convey a lethal imbalance during early embryogenesis. Naturally, if the window of observation were to be shifted to this period of development, more cases would reveal themselves. An example is shown in Figure **5–10**, this being the karyotype from the products of conception obtained at miscarriage in the first trimester from a woman who was herself a translocation carrier, 46,XX,t(13;16)(q12.3;q13). The karyotype of the cultured products, 46,XX,–13,+der(16), displays an overall HAL imbalance of 2.6%. Two previous miscarriages to this couple might possibly also have had this karyotype. Earlier in the piece, at the 3-day embryo stage, selection pressures have not yet come to bear; and thus three embryos at preimplantation diagnosis, all with an adjacent-2 imbalance, as seen from a 46,XY,t(10;18)(q24.1;p11.2) carrier, is perhaps not too remarkable a finding (Munné et al., 2000b).

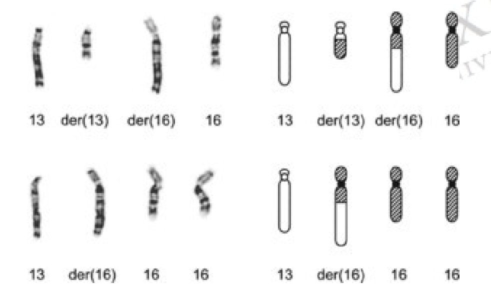


Figure 5–10
Adjacent-2 segregation, with an imbalance lethal in early pregnancy. The mother (*above*) has the karyotype 46,XX,t(13;16)(q12.3;q13). Tissue from the products of conception of a spontaneous first-trimester abortion was cultured, and the chromosomal complement from these cells (*below*) showed the karyotype 46,XX,–13,+der(16). There is monosomy of proximal 13q for a segment of HAL 0.6%, and partial trisomy 16 for a segment of HAL 2.1%. (Case of M. D. Pertile.)

3:1 Segregation with Tertiary Trisomy

Tertiary trisomy is fairly uncommon—or to be precise, fairly uncommonly seen in a term pregnancy—and may arise only when one of the derivative chromosomes is of small content. It exists in the abnormal individual as a supernumerary chromosome, with the karyotype 47,+der. The centric segment will necessarily contain the whole short arm of the derivative chromosome, and it will necessarily be of a chromosome having a small short arm. Almost always, complete long arms (and in fact most complete short arms) contain too much material to allow viability in a supernumerary derivative chromosome, and spontaneous abortion ensues. A rare chance to illustrate this point is given in Fritz et al. (2000) who, as mentioned earlier, studied archived material from an abortus, the mother carrying a subtle translocation, 46,XX,t(4;12)(q34;p13). They showed (by CGH) a tertiary trisomy, 47,XY,+der(4), with almost the entire chromosome 4, and the tip of 12p, present as an additional chromosome. There is, as noted later, a significant maternal age effect in 3:1 imbalance.

Curiously enough, in the most common, by far, human reciprocal translocation, practically all abnormal offspring of the heterozygote have a tertiary trisomy, due to 3:1 meiosis I malsegregation. This is the t(11;22)(q23;q11)[6] (Fig. **5–11a**). The quadrivalent of this 11;22 translocation would have the form outlined in Figure **5–11b**. The content of the smallest chromosome, the der(22), is small (respecting the requirement for the derivative to have a small short arm, chromosome 22 easily qualifies), and its major genetic composition is accounted for by the distal 11q segment. The presence of this 47th chromosome does not necessarily impose a lethal distortion on intrauterine development, and a pregnancy could continue through to the birth of a child who would have trisomy for the segment 11q23-qter (and for the very small segment 22pter-q11), with the karyotype 47,+der(22),t(11;22)(q23;q11). Carter et al. (2009) review the clinical features associated with this imbalance, now known as Emanuel syndrome.



Figure 5–11
Tertiary trisomy. (*a*) The common t(11;22)(q23;q11) in the heterozygous state (*above*) and in the typical unbalanced state (*below*). (*b*) The presumed pachytene configuration during gametogenesis in the heterozygote (chromosome 11 chromatin, open; chromosome 22 chromatin, crosshatched). Arrows indicate movements of chromosomes to daughter cells in a 3:1 tertiary segregation; heavy arrows show the viable trisomic combination.

This t(11;22) is the spectacular exception to the rule that, in different families, translocations arise at different sites. The great majority of families have a "private translocation," and many may represent the first and only case in the whole of human evolution. Apparently, few predispositions for specific rearrangement exist; equally apparently, 11q23 and 22q11 show a remarkable predisposition, which may reflect a physical proximity between the two chromosomes during meiosis (Ashley et al., 2006). Kurahashi and Emanuel (2001) studied normal volunteers, and, being able to test very large numbers of sperm, they could show that de novo t(11;22)(q23;q11) translocations must be being generated from time to time; and Ohye et al. (2010), studying eight de novo cases, showed the translocation in each to have been of paternal origin.

The male t(11;22) heterozygote, at least, does produce other types of unbalanced gamete, as shown on sperm chromosome study (Table 5–1), but none of these is ever viable.[7] Using FISH, Estop et al. (1999) showed in one subject 3:1 segregation in 40% of sperm, and they note a predisposition of this translocation to segregate according to this mode. Data from embryos tested at PGD from t(11;22) carriers are set out in Table 26–1, and these show a much lower frequency of 3:1 embryos from the male carrier (2%), compared with the female (30%), and compared with the 40% sperm rate just mentioned.

Note the point that probands in whom a supernumerary marker chromosome (SMC) is discovered are often found, on parental study, to have a derivative chromosome reflecting a tertiary trisomy (Stamberg and Thomas, 1986). Braddock et al. (2000) describe a family in which an SMC due to 3:1 malsegregation had, initially, escaped recognition as such. A child with "atypical Down syndrome" had been karyotyped as trisomy 21. On attending a Down syndrome clinic at age 9 years, the clinical picture raised doubt and his chromosomes were restudied. He turned out to have a tertiary trisomy for a der(21), which comprised much of chromosome 21 and a small part of distal 5p. His mother and several other relatives carried a t(5;21)(p15.1;q22.1), and a similarly abnormal aunt had the same tertiary trisomy, 47,+der(21). This story has lessons both for cytogeneticists and genetic counselors.

3:1 Segregation with Tertiary Monosomy

If one derivative is very small, and the amount of material that is missing is "monosomically small," the countertype 3:1 22-chromosome gamete may lead to a viable conceptus. Consider the 12;13 translocation t(12;13)(p13.32;q12.11) shown in Figure **5–12a**. The large derivative chromosome is not far from being a composite of the two complete chromosomes. It is missing only subterminal 12p and pericentromeric chromosome 13. This is a "small" loss, and thus the 45,der(12) conceptus is viable (Fig. **5–12b**).

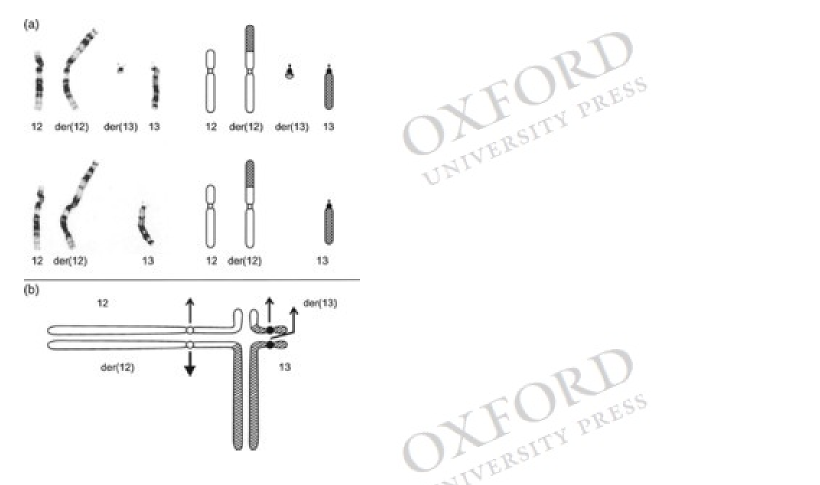


Figure 5–12
Tertiary monosomy. (*a*) Mother (*above*) has a reciprocal translocation between nos. 12 and 13, 46,t(12;13)(p13.32;q12.11). Two children (*below*) inherited the derivative 12, but no normal chromosome 12 or 13 from the mother, and have the karyotype 45,der(12). They are thus monosomic for the tip of 12p and pericentromeric 13 (and only a mildly abnormal phenotype). Chorionic villus sampling in a subsequent pregnancy gave a 46,XX result; an elder sister was a balanced carrier. (Case of M. D. Pertile.) (*b*) The presumed pachytene configuration during gametogenesis in the heterozygote (chromosome 12 chromatin, open; chromosome 13 chromatin, crosshatched). Arrows indicate movements of chromosomes to daughter cells in a 3:1 tertiary segregation; heavy arrow shows the monosomic complement. Alternatively, the three large chromosomes might form a trivalent, and the tiny der(13), being unattached, might segregate at random.

Any initially 45-count karyotype obliges consideration that there may, in fact, be a tertiary monosomy. For example, Courtens et al. (1994) describe an infant who died at birth with, at first sight cytogenetically, monosomy 21 (45,–21). But with FISH and molecular studies, a 45,+der(1) from a maternal 1;21 translocation was discovered.

Sometimes the two phenotypes of the two contributing monosomies can be separately discerned. Thus, Reddy et al. (1996) describe children with a combined Di George (DGS) and Wolf-Hirschhorn (WHS) phenotype, having the karyotype 45,der(4)t(4;22)(p16.3;q11.2)mat. The large derivative chromosome comprised almost all of 4 and almost all of 22q, but it lacked the WHS and DGS critical segments. Similarly, McGoey and Lacassie (2009) give an account of the child of a carrier father who had features of both DGS and subtelomeric 9q deletion, with the karyotype 45,XX,der(9)t(9;22)(q34.3;q11.2)pat. Wenger et al. (1997) report a mother with a t(8;15)(p23.3;q13) whose child had the karyotype 45,der(8) and presented a phenotype with features of Angelman syndrome (due to loss of the maternally originating segment 15q11-q13) and of 8p−syndrome. Torisu et al. (2004) describe a severely retarded, epileptic child with tertiary monosomy dictating a combination of Angelman syndrome and the 1p36 deletion syndrome: her karyotype was 45,XX,der(1)t(1;15)(p36.31;q13.1)mat. An interesting historical example, in that it provided a key observation toward the discovery of the *TSC2* locus, is that of a child with 45,der(16), who had monosomy for the segment 16p13-pter, and who had both tuberous sclerosis and polycystic kidney disease, due to loss and disruption, respectively, of the adjacent *TSC2* and *PKD1* loci. The heterozygous 46,t(16;22) family members had polycystic kidney disease, due to the disruption of *PKD1* (European Polycystic Kidney Disease Consortium, 1994).

However, the great majority of conceptions with a tertiary monosomy are expected to be lethal in utero. A direct demonstration of this circumstance is illustrated in the case of a 3:1 malsegregation of a maternal t(11;22) in a spontaneous abortus at 7 weeks gestation with 45,der(11), which resulted in monosomy for distal 11q and monosomy for proximal 22q (Jobanputra et al., 2005).

3:1 Segregation with Interchange Trisomy

This mode of segregation can only produce a liveborn child when a “trisomically viable chromosome” (i.e., 13, 18, or 21, or even 22) participates in the translocation (Fig. **5–13a**). This chromosome accompanies the two translocation (interchange) elements of the quadrivalent to one daughter cell (Fig. **5–13b**). Interchange trisomy 21 is rare, interchange trisomies 13 and 18 extremely rare, and interchange trisomy 22 barely recorded (Stene and Stengel-Rutkowski, 1988; Teshima et al. 1992b; Koskinen et al., 1993; Patel and Madon, 2004). Concerning other (nonviable) autosomes, there are examples of interchange trisomies 2, 6, and 7, from a translocation parent, these aneuploidies having been identified at PGD, or upon analysis of abortus material (Cockwell et al., 1996a; Conn et al., 1999; Lorda-Sánchez et al., 2005).

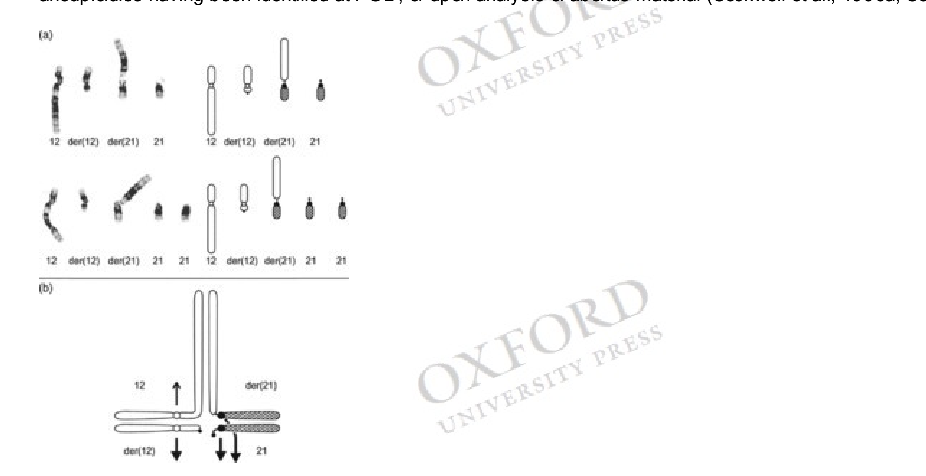




Figure 5–13
Interchange trisomy. (*a*) Mother (*above*) has a reciprocal translocation between nos. 12 and 21; her child (*below*) inherited the maternal translocation chromosomes and a “free” chromosome 21. The breakpoints are 12q13.1 and 21p13; an apparent gap, comprising satellite stalk, can be discerned between the centromere of the der(21) and its 12q component. (Case of R. Oertel.) (*b*) The presumed pachytene configuration during gametogenesis in the heterozygote (chromosome 12 chromatin, open; chromosome 21 chromatin, crosshatched). Arrows indicate movements of chromosomes to daughter cells in 3:1 interchange segregation; heavy arrows show the trisomic combination.

> Theoretically, uniparental disomy can be a consequence of interchange trisomy, if one of the “trisomic” chromosomes is subsequently lost postzygotically, and if this chromosome had come from the noncarrier parent. If this chromosome is one that is subject to imprinting according to parent of origin, phenotypic abnormality will be the consequence, notwithstanding the apparently balanced karyotype, the same as the parent's. Thus, for example, a 46,t(8;15) father could have a 46,t(8;15) child with Angelman syndrome, or a mother a child with Prader-Willi syndrome. Actual examples of this type of mechanism are extremely rare (Kotzot, 2001; Dupont et al., 2002; Heidemann et al., 2010).

3:1 Segregation with Interchange Monosomy

Autosomal monosomy is typically associated with very early arrested development of the embryo, by the blastocyst (day 5) stage. Only with PGD does a practical relevance possibly emerge, since there has not yet been the chance for selection pressure to have operated. In the PGD case reported in Conn et al. (1999) noted earlier, the woman being a t(6;21) heterozygote, a transferred embryo that implanted only transiently may have had an interchange monosomy 6.

Yet to be observed is uniparental disomy following “correction” by duplication of the single normal homolog in the embryo resulting from interchange monosomy. The countertype gamete in Figure **5–13a**, for example, would be nullisomic for 21. Replication of the chromosome 21 from the other gamete could restore disomy and with a normal karyotype. Note that this would be uniparental *iso*disomy and from the *other* parent.

4:0 Segregation

A total nondisjunction of the quadrivalent complex is rare indeed. FISH allows for very large numbers of sperm to be analyzed, and so, in a few cases, a very few (fractions of a percent) 4:0 gametes are seen (Table 5–1). If 4:0 segregation should happen, preimplantation lethality would, in most, be the likely consequence. Out of interest, the reader may care to note how a hypothetical double trisomy of 18 plus 21, based on the 4:0 combination in Figure **5–4** (15), and potentially associated with some in utero survival (Reddy, 1997), could come from the t(18;21) shown in Figure **5–15**. PGD has now brought the 4:0 gamete out from its former place of practical irrelevance.



Figure 5–15
Several viable unbalanced forms. The karyotype is illustrated (*top*) of a mother carrying the translocation t(18;21)(q22.1;q11.2). She had a miscarriage due to adjacent-1 segregation, an abnormal child with a tertiary trisomy, and a stillborn child with a tertiary monosomy, as depicted in the cartoon karyotypes. An uncle with Down syndrome may have had the same adjacent-1 karyotype as in the second row, or possibly interchange trisomy 21, as depicted in the bottom row. (Case of M. D. Pertile.)

More than One Unbalanced Segregant Type

Sometimes a reciprocal translocation has characteristics associated with more than one type of malsegregation; so each type may be seen in the family (Abeliovich et al., 1982). Consider the 11;18 translocation t(11;18)(p15;q11) shown in Figure **5–14**. First, the translocated segments are small: 18q is known to be viable in the trisomic state, and the tip of 11p contributes a minimal/nil imbalance (i.e., this is regarded as a single-segment imbalance). Thus, one of the adjacent-1 segregants is presumed to be viable. Second, two component chromosomes of the pachytene configuration, the der(18) and chromosome 18, are of small overall genetic content. Thus, 3:1 segregation with either tertiary trisomy or interchange trisomy is possible. In the event, the two unbalanced karyotypes in this family reflected adjacent-1 and 3:1 tertiary trisomy segregation. The t(9;21) discussed earlier as an example of adjacent-2 segregation could also, in theory, produce a second viable complement, interchange trisomy 21.

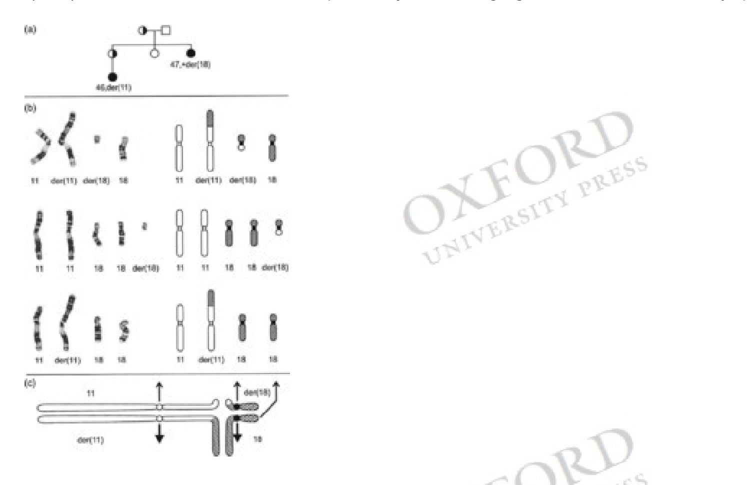


Figure 5–14
More than one viable segregant form. (*a*) Pedigree. Filled symbols, unbalanced karyotype, as shown; half-filled symbols, heterozygote. (*b*) Mother and one daughter have a reciprocal translocation of chromosomes 11 and 18, t(11;18)(p15;q11) (*upper*). Each had one unbalanced offspring, one having 47,+der(18) due to 3:1 tertiary trisomy (middle), and the other 46,+der(11) from adjacent-1 segregation (*lower*). The former had a complete trisomy 18p and the latter a partial 18q trisomy. (Case of C. Ho and I. Teshima.) (*c*) The presumed pachytene configuration during gametogenesis in the heterozygote (chromosome 11 chromatin, open; chromosome 18 chromatin, crosshatched). Heavy arrows indicate one adjacent-1 segregant movement of chromosomes, and light arrows indicate movements of chromosomes to daughter cells in a 3:1 tertiary trisomy segregation, each of which occurred in this family. (From Gardner et al., 1978.)

Rather more spectacular is the translocation illustrated in Figure **5–15**. A mother had the karyotype 46,XX,t(18;21)(q22.1;q11.2): these breakpoints are toward the end of 18q and immediately below the centromere in 21q. She had a stillborn child with tertiary monosomy, a miscarriage with adjacent-1 malsegregation (and two other unkaryotyped miscarriages), and a surviving child with tertiary trisomy. These three karyotyped pregnancy outcomes were, respectively, 45,der(18), 46,der(18), and 47,+der(18). An uncle said to have had Down syndrome may have had the 46,der(18) karyotype (the der(18) includes the segment of 21 that contributes substantially to the DS phenotype), or possibly interchange trisomy with 47,+21,t(18;21). Some of the other possible imbalanced segregants could theoretically be viable, and the reader may wish to determine which ones these would be. This is due to the fact that many of these combinations have a genetically “small” imbalance. All partial trisomies and some partial monosomies for segments of chromosomes 18 and 21 can be viable as a single imbalance; and, when two different imbalances occur in combination, for example, partial trisomy 21 plus partial monosomy 18, a pregnancy may still be capable of proceeding substantially along its course.

No Unbalanced Mode Possible

Finally, for the translocation in which the quadrivalent is characterized by long translocated and long centric segments, no mode of segregation could produce a viable unbalanced outcome. We emphasize the point that many reciprocal translocations (including whole-arm translocations) are in this category. Consider the family depicted in Figure **5–16**, in which a 4;6 translocation t(4;6)(q25;p23) was discovered by chance at amniocentesis. The quadrivalent would have the form depicted in Figure **5–5d**. It possesses none of the criteria that would allow a viable imbalance to result, by whatever mode of segregation. The translocated segments are both large (leading to double-segment imbalance); the centric segments are very large; and the content of all four chromosomes is large. Miscarriage is as far as any unbalanced conceptus could ever get. The large kindred of Madan and Kleinhout (1987) graphically illustrates this circumstance: 11 carriers of a t(1;20)(p36;p11) had had two or more miscarriages, and numerous normal children, but none had an abnormal child. In some such translocations identified fortuitously, for example at amniocentesis for maternal age, there may be little or no

history of apparent reproductive difficulty.

Meiosis II Nondisjunction.

The great majority of segregant forms will have been determined at meiosis I. Meiosis II is not to be completely overlooked, however. A balanced complement may have been transmitted at meiosis I, but a nondisjunction at the following second meiotic division could then produce a gamete with an extra copy, or no copy, of one or other of the derivative chromosomes. In consequence, the conception would have either a partial trisomy of the component parts of the additional derivative chromosome, or a partial monosomy (Masuno et al., 1991). Illustrating the former possibility, Wu et al. (2009) document the case of a father who carried a t(9;15)(q34.3;q13), and whose child, who developed severe autism, had the karyotype 47,XY,t(9;15),+der(15). This imparted a duplication of the proximal long arm of chromosome 15 (which contains autism-susceptibility genes), and of an 8 Mb segment on distal 9q. This type of "secondary nondisjunction" is very rarely observed.[8]

Meiotic Drive.

As well as the effect of in utero survivability discussed earlier, the nature of the quadrivalent may, of itself, influence segregation. The propensity for a particular segregation outcome may reflect a particular geometry of the quadrivalent, and what sort of ring or chain it forms. Quadrivalents that have translocation chromosomes with short translocated segments more usually form a ring and have the quality of being more likely to generate adjacent-1 gametes, while those with short centric segments, more often existing as a chain, may have a predisposition to the formation of adjacent-2 and 3:1 gametes (Faraut et al., 2000; Benet et al., 2005). This predisposition to form particular classes of segregant gamete may be considered a form of "meiotic drive."

As we have had cause to comment more than once, each translocation is entitled to its individuality and need not necessarily follow the "rules" set out earlier. Faraut et al. (2000) identified a few translocations that "should" have produced sperm with certain expected proportions of adjacent-1 and adjacent-2, but which did not. We have seen a remarkable family in which, over some 10 years of marriage, the woman had innumerable very early miscarriages, about eight at 12–14 weeks, one at 16 weeks, and one phenotypically normal son. The husband (and the son) had the translocation 46,XY,t(12;20)(q15;p13). Perhaps, the quadrivalent was configured in such a way that alternate segregation was very difficult to achieve, and so almost all sperm had an unbalanced complement. De Perdigo et al. (1991) report a possibly similar case, in which they propose that heterosynapsis in the quadrivalent permitted spermatogenesis to proceed, but at the cost of producing many unbalanced gametes. In a family reported in Groen et al. (1998) with a mother having the karyotype 46,XX,t(5q;6q), q35.2;q27), seven sequential retarded siblings of hers are presumed to have had a dup(5)/del(6) karyotype, and only the two eldest and the youngest were phenotypically normal. Observations from the PGD laboratory are further illustrating the point that translocation carriers with very poor reproductive histories may indeed reflect a very high rate of meiotic malsegregation. The patient in Conn et al. (1999) mentioned earlier, she having the karyotype 46,XX,t(6;21)(q13;q22.3), had had four miscarriages and one child with interchange trisomy 21. She came to PGD, and not one of two oöcytes and nine embryos were chromosomally normal (mostly 3:1, some adjacent segregations).

Failure to Form Quadrivalent.

Where very small segments are involved, the imperative may lack for the coming together of the four chromosomes with segments in common. This might pertain, for example, to a translocation such as the t(14;15)(q12;q12) in Burke et al. (1996), in which the derivative chromosomes each comprise almost an entire chromosome 14 and chromosome 15, respectively. The 14 and der(15), and the 15 and der(14), might simply synapse as bivalent pairs. The same may very well apply to the general case of the subtelomeric translocation, as discussed earlier. If that were indeed so, then a segregation ratio of 1:1:1:1 would presumably operate, for normal, balanced, and the two imbalanced outcomes: clearly, a high-risk circumstance.

Different grounds for the nonformation of a quadrivalent may exist if one chromosome is a very small one. While the three other chromosomes could have come together as a trivalent, the fourth very small one might fail to be captured by the meiotic mechanism. That being so, it could then segregate at random. This could imply a high risk, and might be the reason, for example, that the t(12;13) carrier mother in Figure **5–12** had two out of her four children with a tertiary monosomy. But this is speculative. Detaching of the small derivative from the quadrivalent is an alternative possibility, as discussed in the next paragraph.

Parental Origin and Parental Age Effect.

There are more women who have been mothers (whether the children are normal or not) than there are men who have been fathers in translocation families. In their review of 1597 children in 1271 translocation families, Faraut et al. (2000) found the mother to be the carrier parent in 61% of the adjacent-1 children, 70% of the adjacent-2 children, and in as many as 92% of the unbalanced offspring from 3:1 segregations. This 3:1 association may reflect an actual maternal predisposition. With advancing maternal age, and after some decades of being held in meiosis I prophase, the small supernumerary chromosome may be increasingly likely to detach from the quadrivalent, and then to migrate at random to one or other daughter cell, when meiosis reactivates in that particular menstrual cycle. On the other hand, no maternal age effect applies to adjacent-1 or adjacent-2 offspring. Here, the maternal excess may more accurately be termed a paternal deficiency, due to reduction in fertility of the male heterozygote (see later discussion). No paternal age effect is discernible in any segregation mode.

The Practical Problem of the De Novo Apparently Balanced Translocation

A not uncommon problem encountered in the genetic clinic is that of the de novo apparently balanced translocation, which has been discovered in the course of investigation of a child with a nonspecific picture of cognitive compromise and sometimes also some dysmorphic signs. Is the translocation causative, or simply coincidental? Families like those reported in Hussain et al. (2000) offer useful illustration: in this example, an apparently balanced translocation that was cosegregating with a phenotype of nonsyndromic mental retardation. Presumably this translocation, a t(1;17)(p36.3; p11.2), had been de novo at some prior point, possibly with the 65-year-old grandmother of their index case. In this family, there were children and grandchildren, seven of them, to bear witness to the harmful role of the translocation. Thus, the point is underlined: at least some apparently balanced translocations are indeed the cause of the nonspecific clinical picture with which they are associated. In many, however, when these translocations have arisen de novo, it will not be possible, based on classical cytogenetics, to state that the link with phenotypic abnormality was causative, or whether simple coincidence might be the explanation.

Newer technologies may now cast light. Using array-CGH with FISH, De Gregori et al. (2007) undertook a systematic search and showed that 40% of 27 apparently balanced de novo translocations in abnormal individuals were, in fact, not so, with microdeletion demonstrable at the sites of breakpoint. In some, they identified de novo deletions at other chromosomal sites than at the translocation breakpoints; and they thus point to the value of microarray in being able to interrogate the whole genome. All 14 prenatally diagnosed de novo translocations in this study were balanced, on this methodology. Baptista et al. (2008) undertook a similar microarray exercise in 31 normal and 14 abnormal cases, making comparison against the absence or presence of genomic imbalance and gene disruption. Some in the abnormal group had microdeletions (although not all were at the breakpoint sites) that offered a likely explanation for the observed phenotype, but none of the normals did. Intriguingly, the frequency of gene disruption was similar in the two groups, implying that a loss of function (presumably) of one copy of some genes may be without phenotypic consequence.

Infertility

Infrequently, the process of gamete formation in the male translocation heterozygote is disturbed to the extent that gametogenic arrest results. In the analysis of reproductive outcomes in the translocation families of Faraut et al. (2000), looking at prenatal diagnoses in order to avoid bias, 61% of all fetuses came from a carrier mother, versus only 39% from a carrier father; this ratio presumably reflects male infertility associated with the carrier state. This infertility is generally not something that is predictable from the nature of the translocation, and indeed the same translocation may compromise fertility in only some men in the family. Presumably there is, in addition, an effect of the genetic background otherwise (Rumpler, 2001).

The detrimental process is considered to be a consequence of failure of pairing (asynapsis or heterosynapsis) of homologous elements in the translocation chromosomes during meiosis I, which promotes association of the quadrivalent with the X-Y bivalent, also known as the sex chromosome vesicle (Paoloni-Giacobino et al., 2000b). The more frequently this association occurs, the more marked the effect upon sperm count. The semen profiles of translocation carriers may not always predict fertility outcomes. In the two cases reported by Oliver-Bonet et al. (2005), one male carried a t(10;14), was normozoöspermic, but had 30% of spermatocytes showing synaptic pairing abnormalities; the other was a t(13;20) man, who was azoöspermic, and showed synaptic pairing abnormalities in 71% of meiotic spreads. This latter carrier also showed decreased recombination frequencies. In men with intact fertility, the spatial organization of chromosomes within the sperm nucleus differs from normal controls (Wiland et al., 2008). Rearranged chromosomes are not able to be packaged as neatly as they should, so to speak; and this might, in some men, be an additional contributory factor compromising spermatogenesis.

The sex difference in susceptibility is striking in the family of Paoloni-Giacobino et al. (2000b). A mother was a t(6;21)(p21.1;p13) heterozygote, and she had eight children, four sons and four daughters (and two miscarriages). The four sons, each one 46,XY,t(6;21), were all married, one three times, and none had any children. Each had severe oligospermia or oligoasthenoteratospermia, and two having testicular biopsies manifested spermatogenic arrest at meiosis I prophase, with extensive asynapsis of several chromosomes. Two sisters were 46,XX,t(6;21), and the one who was married had had two children (and two miscarriages). Oögenesis may not, however, be entirely immune to the translocation obstacle (Speed, 1988; Mittwoch, 1992). Tupler et al. (1994) report two women, one with primary and the other with secondary amenorrhea, who had a balanced reciprocal translocation. Ovarian biopsy in the former, whose translocation was a de novo one, showed absence of the follicle structures in the cortex. Such cases may exemplify a rare translocation effect in the female; equally, given the frequency in the population of the translocation heterozygote, it does remain possible that the link is coincidental rather than causal.

Assisted Reproduction.

Assisted conception may enable infertile men with a translocation to become fathers. But of course the translocation will, in any event, convey a genetic risk. Meschede et al. (1997) report a man with a t(1;9)(q44;p11.2) having intracytoplasmic sperm injection (ICSI), and two embryos were successfully transferred. At prenatal diagnosis, one twin had an adjacent-1 imbalance conferring a 9p trisomy, the other being 46,XX, and the parents chose selective abortion. Belin et al. (1999) describe a triplet pregnancy achieved via ICSI, the father being a t(20;22) heterozygote. Two normal babies were born (one karyotypically normal, one with the translocation), but the third, with a dup(20p)/del(22q) imbalance, was severely malformed and died in the neonatal period. Meschede et al. (1997) suggest that the population of fertilizing sperm from carriers may differ between ICSI and natural conceptions, and that the risk criteria generally set forth in this chapter should be used with caution in couples needing ICSI. Could a chromosomally unbalanced but morphologically normal sperm have a better chance of being selected at ICSI than of succeeding in the open competition that obtains in vivo? We have yet to learn.

Rare Complexities

Translocations with Breakpoints at Vital Loci.

The great majority of breakpoints in familial translocations between autosomes are apparently sited at points within the genome where they have no effect on its smooth running. Thus, the balanced carriers are phenotypically normal. Rarely, the act of breakage and reunion might compromise a gene or genes; naturally, this is particularly observed in de novo, not familial cases. Compromise could be due to disruption or deletion of genes, or it might reflect a position effect.

A detailed example of gene disruption is provided in Kurahashi et al. (1998). A child with lissencephaly (a severe structural brain abnormality) had a t(8;17)(p11.2;p13.3), in this case de novo. The p13.3 breakpoint on the chromosome 17 was sited within intron 1 of the *LIS* gene, with the gene being split between the two derivative chromosomes: its 5' part on the der(8), and the rest of it on the der(17). The gene could not, in consequence, function. A similar mechanism is likely the basis of the cognitive and motor delay, and tracheo-esophageal atresia, in a girl with a t(6;15) studied by Giorda et al. (2004), the disrupted gene being the muscle and brain specific gene *BPAG1*. In the extraordinary coincidence of a recessive mutation on the intact homolog, a translocation breakpoint that disrupted a gene would lead to the appearance of the recessive syndrome, as Kuechler et al. (2010) exemplify in a teenage girl with gonadal failure, who received an apparently balanced t(2;8)(p21;p23.1) from her mother that removed two exons from the *FSHR* gene (FSH receptor gene, which is located at 2p21), and a point mutation in that same gene on her paternal chromosome 2.

As for the position-effect scenario, there are numerous examples, in which a specific phenotype is caused due to a close-by intact gene failing to function. We illustrate in Figure 19–12 (in Chapter 19) one of the earliest such cases, due to a chromosome 17q25.1 translocation whose breakpoint is 50 kb away from the *SOX9* locus, leading to campomelic dysplasia (Wagner et al., 1994). More recent cases include a translocation with an 11p13 breakpoint that translocates the *PAX6* gene into a chromosomal environment which does not permit its normal expression, with consequential abnormal development of the iris (aniridia); a girl with severe speech impairment who had a t(7;10)(q31;p14) influencing the *FOXP2* language-acquisition gene (see later) at 7q31; and a t(12;17)(p13.3;q21.3) affecting the function of the *HOXB* gene cluster, causing mental retardation and skeletal malformations (Crolla and van Heyningen, 2002; Yue et al., 2007; Kosho et al., 2008).

A salutary tale comes from the study of a family with an apparently dominantly inherited syndrome of skeletal anomalies, in which previous cytogenetic tests had given normal results (Stalker et al., 2001). Only after the birth of an infant with severe multiple malformations with an unbalanced karyotype was the fact revealed of a balanced t(13;17)(q22.1;q23.3) cosegregating with the phenotype of the syndrome in the family. There is a fair case for considering that a "bone locus" at 17q23.3 had been disrupted or otherwise influenced by the translocation. Stalker et al. rightly comment that a chromosome test is always worth doing in the investigation of an apparently new familial syndrome, earlier reports of normal cytogenetics notwithstanding, especially if the original laboratory material is not available for review. However, there does always remain the simple possibility that a breakpoint and a disease locus are closely linked, and so the translocation and the disease cosegregate in the family (Hecht and Hecht, 1984).

Constitutional translocations might convey a risk for cancer if, for example, a tumor suppressor gene is disabled, or an oncogene is separated from its controlling region. Translocations possibly implying risks for breast cancer, renal cancer, neurofibromatosis type 2, and hematological malignancy are noted in the section on "Genetic Counseling."

Using Translocations to Track Genes.

Translocations associated with mendelian disease can serve as a very helpful signpost in mapping the gene. The t(16;22) segregating in a family with polycystic kidney disease mentioned earlier enabled this gene, *PKD1*, to be isolated: the breakpoint was sited within the gene. Neurofibromatosis type 1 and Sotos syndrome are other common genetic conditions to have been mapped this way (Ledbetter et al., 1989; Kurotaki et al., 2002). Less readily tractable is a condition such as manic-depressive disease (bipolar disorder), in which an incompletely penetrant mendelian gene is a suspected contributory cause. Baysal et al. (1998) studied a family in which five persons in three generations were affected, each of whom carried (as did some unaffected relatives) a t(9;11)(p24;q23.1). A detailed molecular dissection failed to find disruption in any of a number of plausible candidate genes within the breakpoint regions of 9p or 11q; and so the question remains open whether segregation of the translocation with bipolar disorder was causal or coincidental.

When two cases are reported of a mendelian disorder both having a translocation with one breakpoint in common, naturally the case is very much stronger than with a single observation. Thus, there is merit in the proposition of McGhee et al. (2000) that the Coffin-Siris syndrome locus must be at 7q32-q34, they having a patient with a de novo apparently balanced t(7;22)(q32;q11.2), and there being another child on record who had a de novo t(1;7)(q21.3;q34).

A formal review of the usefulness of apparently balanced rearrangements in leading the way to locus discovery is given in Bache et al. (2006). The Danish national register of translocations provided a large database, going back to 1968, from which to seek possible disease associations, both mendelian and of complex genetics. Earlier work from this group is outlined in Table 5–3, a listing of breakpoints associated in more than one case of mental retardation; some of these loci may harbor genes important for brain development.[9] A similar exercise is proceeding in the United States, focusing more on associations of multiple malformations and mental retardation, and enabling these

workers to identify new genes (Higgins et al., 2008).

Table 5–3. Breakpoints in De Novo Rearrangements Associated with Mental Retardation with or without Multiple Congenital Malformations, Observed in More Than One Patient, from a Large Scandinavian Database

1q31	4q35	9p24
1q44	5q13	9q34
2q23	6q27	12q15
4p16	7q22	13q34
4q22	7q36	14q32

Notes: Some of these breakpoints may indicate the sites of disease genes. The 15 breakpoints were seen in 39 patients among 216 with a "disease associated balanced chromosome rearrangement" discovered in 71,739 cytogenetic analyses.

Source: Data from Bugge et al. (2000).

Carrier Couple.

Since reciprocal translocation heterozygotes are not uncommon in the population, on rare occasions both members of a couple will, by chance, carry a translocation (Neu et al., 1988b). We have seen, for example, a couple who had had several miscarriages, from 5–9 weeks gestation. The husband's karyotype was 46,XY,t(7;11)(q22;q23) and the wife's 46,XX,t(7;22)(p13;q11.2). Presumably, their history of miscarriage reflected at least one parent transmitting, with each pregnancy, an unbalanced gamete: rather many unbalanced karyotypes, as the reader can determine, are possible! A normal child is possible if each contributes a normal or a balanced gamete to the same conceptus. It should, in theory, be reasonably likely in a given conception for the two contemporaneous gametes to have arisen from alternate segregation—as an educated guess, the chance might be about 20%—although at the time of our seeing this family only miscarriage had occurred. A child of theirs having each parental translocation would qualify as having a "complex chromosome rearrangement," and we shall follow that case in Chapter 12 (and see Fig. 12–6).

If a translocation is in a family, and a couple are related, the possibility is open that they might both be carriers. Such a circumstance is illustrated in Kupchik et al. (2005), who report a husband and wife with the karyotypes 46,XY,t(16;18)(p13.3;p11.2) and 46,XX,t(16;18)(p13.3;p11.2). Their child received two copies of the der(18) and one of the der(16), due to alternate segregation in one parent and adjacent-1 in the other. As the reader may determine, the end result was a duplication of distal 16p and a deletion of 18p. In Martinet et al. (2006), a first cousin couple each carried a t(17;20)(q21.1;p11.21), and their severely malformed fetus was homozygous for the translocation. The phenotype may have been due to a recessive gene or genes. Similar scenarios with respect to a Robertsonian translocation, and to an inversion, are noted in Chapters 7 and 9, respectively.

Mosaicism.

Almost all balanced reciprocal translocations are seen in the nonmosaic state. This reflects either that the translocation had been inherited from a carrier parent, or that the rearrangement had arisen preconceptually, in one or other gamete. Rarely, a balanced translocation can be generated as a postzygotic event, and the person is a 46,t/46,N mosaic. In a literature review, Leegte et al. (1998) recorded 29 such cases. One of their subjects, for example, was a man who had presented with infertility, and he had the balanced karyotype 46,XY,t(9;15)(q12;p11.2). His mother had this translocation in a minority of cells on peripheral blood analysis, with the karyotype 46,XX,t(9;15)(q12;p11.2)/46,XX; thus, she was revealed as a somatic-gonadal mosaic. Wang et al. (1998) report a mother mosaic for a whole arm translocation, 46,XX,t(10q;16q)/46,XX, who had a child with a presumed uniparental disomy 16 phenotype from postzygotic "correction" of interchange trisomy 16. The first example of mosaicism for a cryptic translocation is reported in Dupont et al. (2008).

Gametic Complementation.

Coincidentally abnormal gametes coming from each parent (Fig. 3–6c in Chapters 3) is an extremely rare observation. Park et al. (1998b) describe a unique example of this scenario in the context of a parental translocation. A father with a balanced t(3;15)(p25;q11.2) transmitted a sperm from adjacent-1 segregation, the der(15) being a very small chromosome. This would have led to a near-complete monosomy 15 in the conception. But this was "corrected" by the mother's transmitting an egg with disomy 15, most probably from a meiosis I error. The child had a partial trisomy for the very small segments 3p25-pter/15pter-q11.2 due to the der(15) as a 47th chromosome, but the apparently typical Prader-Willi clinical picture spoke for the predominant contribution to his phenotype deriving from the maternal uniparental disomy 15.

Unstable Familial Translocation.

Tomkins (1981) documents a family in which a mother with 46,XX,t(11;22)(p11;p12) had one daughter with the same translocation, and another daughter with 46,XX,t(11;15)(p11;p12), and a very few other similar cases are on record. Typically, the translocation breakpoints are at telomeres, centromeres, or in nucleolar organizing regions. There is some sequence similarity in these regions between different chromosomes, and this may set the stage for these very rare "second translocation" events (and see section on "Jumping Translocation," p. 159).

Genetic Counseling



The counselor may have to deal with these questions:

1. Is there a risk of having an abnormal child?
2. If so, what is the magnitude of the risk?
3. What would be the abnormality, and would the child survive?
4. What if the same translocation that I have is found at prenatal diagnosis?
5. What is the risk for pregnancy loss through abortion? Is pregnancy possible?
6. Anything else I should know?

Does a Risk Exist of having an Abnormal Child?

If a family is ascertained through a liveborn aneuploid child, that very fact demonstrates viability for that particular aneuploid combination. It could happen again.

If, on the other hand, the family was ascertained by miscarriage or infertility, or fortuitously, and there is no known family history of an abnormal child, the picture is less clear. Most likely, no aneuploid combination is viable. Alternatively, a viable imbalance may be possible, but it has not yet happened; or an imbalance could occasionally be

viable, but usually it is not, and (so far) has led only to abortion. The approach, here, is to determine the potentially unbalanced segregant outcomes, according to the favored mode of segregation—adjacent-1, adjacent-2, or 3:1—and check to see whether any is on record in a pregnancy that produced an abnormal child. Valuable sources of information are Schinzel's catalog (2001), the European Cytogeneticists Association register of unbalanced chromosome aberrations (ECARUCA), and the U.K. Chromosome Abnormality Database (UKCAD).

Where a single-segment imbalance is a potential outcome in a conceptus, from adjacent segregation, and if the potential imbalance comprises an aneuploidy equal to or less than one of these segments on record, viability must be assumed to be possible. If the potential imbalance comprised an aneuploidy greater than any on record, viability would be unlikely, especially if the aneuploidy is much greater. The great majority of double-segment imbalances from adjacent segregation due to a translocation, ascertained other than by a liveborn aneuploid child, would be expected to lead to lethality in utero. Nearly always, a new double-segment exchange presenting at the clinic will truly be new, and there will be no literature record of exactly the same thing to which the counselor may appeal. Some tertiary trisomies from 3:1 segregation are listed in Schinzel's catalog, or in ECARUCA or UKCAD, but in many instances one has to make an educated guess, erring on the side of caution, whether the combination of partial trisomies from a derivative chromosome might, in sum, be viable.

The Magnitude of Risk

If, in a family, it is judged that there does exist a risk to have an abnormal child, a broad estimate of the level of risk may be derived from a consideration of these factors: the assessed imbalance of potentially viable gametes; the predicted type of segregation leading to potentially viable gametes; the mode of ascertainment of the family; and in 3:1, the sex of the transmitting parent. Most risk figures fall in a range from 0% to 30%; higher risks are rare. These percentages are expressed in terms of abnormal live births as a proportion of all live births, although there are other ways of looking at the risk (see section on “Risk at Time of Prenatal Diagnosis”, and Table 4–1). Overall, the risk is higher in cases ascertained through an abnormal child, versus those identified through other routes; in the review of Youings et al. (2004), the respective pooled figures were 19% and 3%.

A precise risk estimate needs to be based on the actual cytogenetic imbalance. Different chromosomal segments contain, of course, different genomic information. It is scarcely possible to come up with a unifying format, given that chromatin is not uniform; as Cohen et al. (1994) comment, “it would be hazardous to suggest a simple mathematical relationship between unbalance length and viability.” Some segments, in the trisomic state, impose a lesser degree of compromise on the process of embryonic development; such as, for example, 18p, and distal 5p. Other segments, although they may be of shorter length, are lethal during early pregnancy and lead to miscarriage. Some translocations can have their own peculiar segregation characteristics, which a priori were quite unpredictable.

Nonetheless, it is interesting to attempt a correlation of quantitative chromatin imbalance with risk to have a liveborn affected child. Daniel et al. (1989), Cans et al. (1993), and Cohen et al. (1994) have compared the haploid autosomal length (HAL) with viability in translocation families. Most (96%) viable imbalances comprise up to 2% monosomy, and up to 4% trisomy, with combinations of monosomy/trisomy viable only when the additive effect of x% monosomy plus y% trisomy falls within a triangular area defined by joining the 2% and 4% points on the x and y axes of a graph (Fig. **5–17**). A few (4%) fall outside of this area, and these cases define the boundaries of a “surface of viable unbalances,” reflecting the effects of qualitative differences in different segments of chromatin.

Only in the case of recurrent rearrangements does the potential exist for direct extrapolation between families. The representatives of this tiny group are the t(11;22)(q23;q11), of worldwide distribution, mentioned numerous times in this chapter, and common enough that its typical unbalanced form has acquired an eponymous nomenclature, Emanuel syndrome (Carter et al., 2009), and of orders of magnitude less frequent, the t(5;11)(p15;p15) associated with Beckwith-Wiedemann syndrome (Slavotinek et al., 1997), the t(4;8)(p16;p23) (Giglio et al., 2002), and the t(4;11)(p16.2;p15.4) (Thomas et al., 2009).

For routine practice in the genetic clinic, we suggest starting off with the unvarnished empiric data for individual chromosome segments collected by Stengel-Rutkowski and colleagues, as set out in their invaluable monograph (Stengel-Rutkowski et al., 1988), and discussed in a review and further illustrated in practice (Stene and Stengel-Rutkowski, 1988; Midro et al., 1992), and to which we have already referred several times earlier. The figures set out in Tables 5–4, 5–6, and 5–7, for the three major categories of malsegregation, are summarized from their monograph, and from additional subsequent data. It will generally give a false sense of precision to use decimal points; a rounded figure will suffice. The paucity of information for some chromosomes has necessitated lumping of data for considerable lengths of a chromosome arm; the risk figures derived in this way are, naturally, composites, and indicative rather than definitive. We assume that, in different families with (apparently) the same translocation, the genetic risks will likely be the same, regardless of what may have been the mode of ascertainment. And of course, the principle always applies: if the counselee's family is large enough, do a segregation analysis to derive a “private” recu rrence risk.

Table 5–4. Specific Risk Figures, Based upon Empiric Data, for Having a Liveborn Aneuploid Child, or a Child Stillborn or Dying as a Neonate,[a] because of *Single-*Segment Imbalance from 2:2 Adjacent-1 Segregation[b]

TRANSLOCATED SEGMENT THAT WOULD BE IMBALANCED[c]		RISK		
		% LIVEBORN	S.D.	+ % STILLBORN, NEONATAL DEATH[e]
1.	1pter→1p11–34	0		
	1p35	?		
	1qter→q11–22	0		
	q23–32	<1.3		+ 5.1
	q42	13.6	5.2	
2.	2pter→p11–12	0		
	p13–16	<2.5		+ 15.0
	p21–23	5.7	3.9	+ 14.3
	2qter→q11–23	0		
	q31–32	<1.7		+ 6.7
	q33	20.0	8.9	

	q33–35	22.9	7.1	+ 11.4
3.	3pter→p13–14	0		
	p21	<2.3		+ 13.6
	p22–25	28.6	17.1	
	3qter→q12–13.2	0		
	q21–27	<1.1		
4.	4pter→p11	7.7	5.2	+ 38.5
	p14	15.4	4.5	+ 7.7
	p15	28.6	12	+ 7.1
	4qter→q11–13	?0		
	q21–34	0.8	0.8	+ 14.1
5.	5pter→p11–12	3.3	2.3	+ 13.1
	p13	7.0[d]	2.6	+ 4.0
	5qter→q13–21	?		
	q22–33	7.7	7.4	+ 7.7
	q34	25.0	7.2	
6.	6pter→p11–12	?		
	p21.2–24	1.3	1.3	+ 11.8
	6qter→q11–16	?0		
	q21–24	20.0	17.9	
	q25–26	33.3	15.7	+ 33.3
7.	7pter→p11–13	4.4	3.0	+ 4.4
	p15–21	19.1	8.6	+ 4.8
	7qter→q11–21	?0		
	q22–35	<0.8		+ 7.9
8.	8pter→p11–23	9.1	3.5	
	p23.1	40[e]	12.6	+20
	8qter→q11–13	2.0	2.0	
	q21.2–24.2	11.1	6.1	
9.	9pter→p11.2	11.8	3.7	+9.2
	p13	25	8.8	+4.2
	p22	21.2	4.4	+2.4
	9qter→q11–13	0		
	q21–33	<0.8		+ 8.3
10.	10pter→p11.1	4.7	2.6	+ 4.7
	p12–14	18.8	9.7	+ 18.8

	10qter→q11–21	?0		
	q22–23	<1.4		+ 5.7
	q24	5.9	2.6	+ 9.4
	q25–26	14.0	4.9	+ 12.0
11.	11pter→p11–13	?0		
	p14	<3.1		+ 6.3
	11qter→q13–22	<2.6		
	q23	7.0	3.9	+ 18.6
12.	12pter→p11.1	9.4	5.2	+ 3.1
	p12	9.1	8.7	+ 18.2
	12qter→q11–15	0		
	q21–24.3	<1.5		+ 3.8
13.	13qter→q21–32	1.6	1.1	
14.	14qter→q11.1–31	1.0	1.0	
15.	15qter→q11–15	0		
	q21–25	2.7	2.7	
16.	16pter→p11.11	8.3	3.6	
	16qter→q11–13	6.2	6	+ <3.1
	q21–23	<5.4		+ <5.4
17.	17pter→p13.3	18.9	3.5	+ 7.1
	p11.1	<2.7		
	17qter→q11–12	?0		
	q21–23	10.0	6.7	
18.	18pter→p11.1–11.2	? (probably high)		
	18qter→q11.1–12	2.5	2.5	
	q21	2.9	2.8	+ 6.7
	q22	15.0	7.8	+ 15.0
19.	19pter→p11–13.2	?0		
	19qter→q11–12	?0		
	q13.2–13.3	11.1	6.1	
20.	20pter→p11.1–11.2	20.0	8.0	
	20qter→q11.1	?0		
21.	21qter→q11.1–22	13.8	6.4	
22.	22qter→q11.1–13	<2.6		

[a] Figures are expressed as a percentage of all karyotyped liveborn infants, typically considered as a baby of >28 weeks gestation, with at least survival beyond the neonatal period. Where there are data relating to unkaryotyped stillbirths or neonatal deaths, the figures for these are indicated with a + sign in the third column under "Risk," as a probable additional component of the overall risk, on the assumption that many, at least, of these cases would have been karyotypically abnormal. The maximum estimate of risk will thus be given by the sum of the two percentage figures. This combined figure may be an overestimate, but if so, likely of small degree; and this may

be the more useful figure to consider.

[b] One specific translocated segment is of substantial genetic content (the one shown here), and the other is judged to be of minimal content. For adjacent-1 segregation, the risk does not differ between male and female heterozygotes. For segments not listed here, no specific data are recorded in Stengel-Rutkowski et al. (1988).

[c] Some segments are noted precisely (e.g., 1pter→1p35). Most are given as a pair of breakpoints encompassing a range (e.g., 1pter→1p11–34), extending from a maximum length of terminal-to-proximal breakpoint, to a minimum length of terminal-to-distal breakpoint. Thus, 1pter→1p11–34 refers to an imbalanced segment comprising anywhere from a maximum of 1pter→1p11 (the whole of the short arm) to a minimum of 1pter→1p34 (about one-third of the short arm).

[d] In one reported large family with several cases of "pure" deletion or duplication of this segment (the other segment being derived from acrocentric short arm), the risk was very high: 54% (De Carvalho et al., 2008, and see text).

[e] When the combined live birth + neonatal death figure approximates 50%, this may suggest that the single-segment imbalance is fully viable in utero in either the duplicated or deleted state, with approximately equal numbers of offspring due to alternate and to adjacent-1 segregation.

S.D., standard deviation; ?, rare cases have occurred, but data too few to derive a figure;?0, probably no risk; <, no additional aneuploid child has been born apart from the proband, figure is estimate of upper limit of risk interval.

Source: From Stengel-Rutkowski et al., 1988, with further entries/amendments from Pollin et al. 1999 (17p13.3), Stasiewicz-Jarocka et al. 2000 and 2004 (1q42, 2q33, 16q), and Panasiuk et al. 2007 and 2009 (4p, 9p), and personal communication from A. Midro 2009 (8p23.1).

Table 5–6. Specific Risk Figures for Liveborn Aneuploid Child due to Imbalance from 2:2 Adjacent-2 Segregation

CENTRIC SEGMENT THAT WOULD BE IMBALANCED		RISK	
		%	S.D.
4.	4pter→q11–13	?0	
8.	8pter→q12–13	?	
9.	9pter→q11–13	18.4	4.5
10.	10pter→q11–21	?	
12.	12pter→q11–13	?	
13.	13pter→q14–21	?	
14.	14pter→q21–22	?	
15.	15pter→q13–24	11.8	7.8
20.	20pter→q11.1	27.3	13.4
21.	21pter→q11.1–22	?	

Note: Figures are expressed as a percentage of all live births. No obvious difference exists according to sex of parent. For segments not listed, no specific data are recorded in Stengel-Rutkowski et al.

?, rare cases have occurred, but data too few to derive a figure; ?0, probably no risk.

Source: From Stengel-Rutkowski et al. (1988).

Table 5–7. Specific Risk Figures for Liveborn Aneuploid Child due to Imbalance from 3:1 Single-Segment Segregation

A. Tertiary Trisomy or Monosomy			
SEGMENT THAT WOULD BE IMBALANCED		**RISK**	
		%	S.D.
4.	4pter→q12–13	?	
8.	8pter→q12–13	?	
9.	9pter→q11–32	1.7 (mat)	1.7
		?0 (pat)	
10.	10pter→q11.1–21	?	
11.	11qter→q23*	3.7% (mat)	

		<0.7% (pat)	
12.	12pter→q11–13	?	
13.	13pter→q12–33	2.6 (mat)	1.8
		0 (pat)	
14.	14pter→q11.1–24	2.6 (mat)	2.6
		<0.8 (pat)	
15.	15pter→q11.1–24	<0.9	
16.	16pter→p11.1	<1.8 (mat)	
		0 (pat)	
	16qter→q11.1-p11.1	? (mat)	
18.	18pter→p11.1–21	<1.3 (mat)	
		0 (pat)	
20.	20pter→q11.1	<4.4 (mat)	
		?0 (pat)	
21.	21pter→q11.1–22	6.9 (mat)	4.7
22.	22pter→q11.1–13	<3.5 (mat)	
		? (pat)	

B. Interchange Trisomy

CHROMOSOME THAT WOULD BE TRISOMIC		**RISK**
	%	S.D.
13	<0.2 (mat)	
	0 (pat)	
18	<0.2 (mat)	
	<0.3 (pat)	
21	0.5 (mat)	0.5
	<0.6 (pat)	

Notes. Figures are expressed as a percentage of all live births. Risks for maternal transmission (mat) are typically greater than for paternal (pat) in 3:1 segregations. For segments not listed, no specific data recorded in Stengel-Rutkowski et al.

?, rare cases have occurred, but data too few to derive a figure; ?0, probably no risk.

* The common t(11;22)(q23;q11); see p. [link].

Source: From Stengel-Rutkowski et al. (1988).

The figure given for a segment, say, q31/q34-qter—in other words, a lumped figure applying to a segment extending anywhere from q31-qter to q34-qter—might be given as <0.8%: in other words, a very small risk. (The "less than" sign in the risk data tables is used for estimates in those translocations where no additional aneuploid child has been born apart from probands.) But this figure might have been based mostly on data from families having a q31 breakpoint. A breakpoint at q34 might happen to exclude a dosage-sensitive region of major effect within q33, and thus imbalance for the slightly smaller segment q34-qter might be of considerably greater viability. The risk figure needs to be interpreted intelligently in the light of what is otherwise known from the literature about the segments in question (consult Schinzel, ECARUCA, or UKCAD), and naturally from observation within the same family.

The reader consulting and using these figures, imperfect though they may be, will gain a good sense of the practical principles of estimating risk. New data may come to hand. For example, Stasiewicz-Jarockaa et al. (2004) assembled data from 65 new pedigrees involving 16q, to add to the original 35 pedigrees from Stengel-Rutkowski et al. and the new risk calculations are included in Tables 5–4 and 5–5. As expected, the new data continue to be consistent with the notion that the risk for unbalanced offspring increases with decreasing length of the segments. In another study, the methods of Stengel-Rutkowski et al. were applied to a large pedigree segregating a double-segment t(7;13)(q34;q13), together with a sperm karyotype analysis. Midro and colleagues (2006) were able to predict the chance of a miscarriage or stillbirth from carriers in this family to be 13% and 30%, respectively, whereas direct examination of sperm karyotypes indicated 60% abnormal sperm. The high rate of selection against abnormal

karyotypes, applying in particular in the latter part of pregnancy with this particular translocation, resulted in a very low presumed risk (0.3%) of the abnormal outcome of a surviving liveborn (and see Table 5–5).

Table 5–5. Risk Figures for Having a Liveborn Aneuploid Child, or a Child Stillborn or Dying as a Neonate,[a] because of *Double*-Segment Imbalance from 2:2 Adjacent-1 Segregation, in 13 Specific Translocations[b]

TRANSLOCATIONS	RISK[c]		
	% LIVEBORN	S.D.	+ % STILLBORN, NEONATAL DEATH
t(1;2)(q42;q33)	6.8		
t(1:3)(q42.3;p25)	63.6	14.5	
t(2;13)(p25.1;q32.3)	14.5	7.6	+4.8
t(3;10)(p26; p12)	24.0	8.5	
t(3;15)(q21.3;q26.1)	?0		+17
t(4;5)(p15.1;p12)	1.6		
t(4;8)(p16.1;p23.1)	15		
t(4;9)(p15.2;p13)	3.2	3.2	+ 6.5
t(7;9)(q36.2;p21.2)	30	14.5	+10
t(7;13)(q34;q13)	0.3*		+ 29.0
t(12;14)(q15;q13)	?0		+?0
t(16;19)(q13;q13.3)	1.2		
t(16;20)(q11.1;q12)	1.1		

* Plus another 0.2% to account for a theoretical risk for interchange trisomy 13. The considerable gap to the next risk figure, 29%, reflects the several instances in this family of unkaryotyped stillbirths and early neonatal deaths.

[a] Figures are expressed as a percentage of all karyotyped liveborn infants, typically considered as a baby of >28 weeks gestation, with survival at least beyond the neonatal period. Where there are data relating to unkaryotyped stillbirths or neonatal deaths, the figures for these are indicated with a + sign in the third column under “Risk,” as a probable additional component of the overall risk, on the assumption that many, at least, of these cases would have been karyotypically abnormal. The maximum estimate of risk will thus be given by the sum of the two percentage figures. This combined figure may be an overestimate, but if so, likely of small degree; and this may be the more useful figure to consider. ?0 indicates probably no risk, albeit that the 17% risk figure above for stillbirth/neonatal death in the t(3;15)(q21.3;q26.1) indicates viability of the unbalanced state through to the end of pregnancy.

[b] Families published in Kozma et al. (2004), Midro et al. (2000, 2006), Nucaro et al. (2008), Stasiewicz-Jarocka et al. (2000, 2004), Tranebjaerg et al. (1984), and Wiland et al. (2007), and personal communication of A. Midro (2009).

[c] Some figures come from direct segregation analysis, and in others, from applying this rule: halving the risk for the lesser of the two risks, which would otherwise have applied to each translocated segment when viewed as a single-segment imbalance (see text).

Another tool is “HC Forum” provided on the Internet by Cohen et al. (2001), at http://www.hcforum.net, which uses computerized translocation data from their own and others' material. This can be used as a helpful check on the correctness of the counselor's pachytene diagram and listing of imbalanced gametes. Some risk estimates from one or other of these sources may need to be treated with reservation,[10] and the counselor should always make his or her own judgment based on first principles, and upon an intelligent assessment of the literature relevant to a particular potential imbalance.

A very few translocations, or at least some breakpoints, occur with sufficient frequency that specific risk data can be derived. The obvious example is the recurrent t(11;22). Enough 17p13 translocations were on record to enable Pollin et al. (1999) to work out a group risk figure for distal 17p trisomy or monosomy (the latter producing Miller-Dieker syndrome). The 17p segments ranged in size from 0.12% to 0.38% of HAL, while the segment from the other chromosome was 0.12% to 0.67%, and the combined trisomic/monosomic fractions from 0.24% to 0.9% of HAL. The risks were rather high: 19% for a child with an unbalanced karyotype, rising to 26% if miscarriage and stillbirth were included.

Individual circumstances for different types of predisposing translocations are discussed later. The lowest risk for a surviving abnormal child, namely zero, applies in the case of imbalances of large genetic content, in which in utero lethality would be seen as inevitable; and in families interpreted as being in this category, prenatal diagnosis could be seen as unnecessary (Vauhkonen et al., 1985) or could at least be confined to noninvasive ultrasonography. This essentially no-risk circumstance may apply to a considerable, perhaps the great majority of “translocation couples”.

The historic data we presently have on record, and to which we appeal in deriving risk figures, were based on classical karyotyping, of differing degrees of precision. Some of the breakpoints given might be arguable, the more so from older reports, and in some (probably only a few), an inaccurate designation might materially misrepresent the risk assessment. In the future, new data will increasingly be generated from microarray analyses, which would, in principle, allow for a very precise comparison with families attending the clinic (Fig. **5–18**).

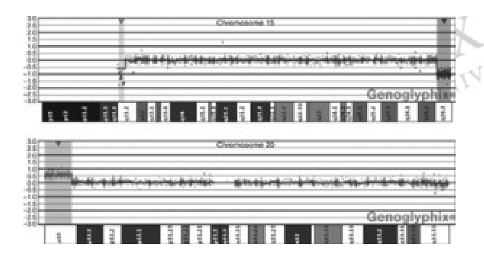


Figure 5–18
Microarray result suggestive of an unbalanced translocation. In each of the figures, probes are ordered on the x-axis according to physical mapping positions for each chromosome. The y axis shows DNA relative amount at the points defined by each oligonucleotide probe: normal diploid amount is at baseline; duplication is above the baseline, and deletion below. Plot areas are shaded pink for gains and blue for losses. In this example, the patient is duplicated (trisomic) for the segment comprising nucleotides 28,197 through 4,174,609 on chromosome 20 (~4.1 Mb), and deleted (monosomic) for the segment comprising nucleotides 96,846,535 through 100,200,997 on distal chromosome 15 (~3.3 Mb). Note that there is also a common deletion variant at proximal 15q. The array nomenclature is arr cgh 15q26.3(96,846,535→100,200,997)×1,20p13(28,197→4,174,609)×3 mat. A microarray analysis only shows gains and losses, and visualization by fluorescence in situ hybridization (FISH) or banding is necessary to determine that this is an unbalanced translocation. Also of note, a microarray analysis on the balanced carrier mother would not have shown the translocation.

Risk at Time of Prenatal Diagnosis.

The risk of detecting an abnormality is higher at prenatal diagnosis than it is at the birth of a live baby. This is because there is differential survival throughout pregnancy. Very unbalanced conceptions will abort before the time of prenatal diagnosis. Daniel et al. (1988) derived an overall figure of about 25% for carriers to have an unbalanced fetal karyotype detected at amniocentesis when ascertainment was through a previous aneuploid child, and about 5% when it was through recurrent miscarriage. The amniocentesis figure is at its highest, 35%, in the carrier whose risk otherwise to have an aneuploid live birth lies in the “medium” range (5%–10%) (Stengel-Rutkowski et al., 1988). To give an example from a specific chromosomal segment, Stengel-Rutkowski et al. record a 6% risk for an imbalance in the liveborn from translocations with a proximal 9p breakpoint, versus a 33% risk to detect an imbalance at amniocentesis. In a series of 57 pregnancies in 40 translocation couples, Barišić et al. (1996) determined an overall risk of 16% to discover an unbalanced karyotype at second-trimester amniocentesis, confirming a higher risk (32%) for couples who had previously had an abnormal child, versus a lower figure (12%) where ascertainment had been because of miscarriage.

The counselor should be clear about these different types of risk figure being offered (Table 4–1). Ultrasonography can be used as an adjunctive procedure, with normal nuchal translucency in the first trimester and absence of structural anomalies in the second trimester predicting a normal/balanced karyotype (Sepulveda et al., 2001).

Risks According to Likely Segregation Mode

Adjacent-1 Segregation, Single Segment

Specific risk figures for individual single-segment imbalances are set out in Table 5–4. A notable point is the number of risk figures that are very small, less than 1%. This most likely reflects that many imbalances are almost always lethal in utero, and survival through to term is the exception. In fact, we can say that, in order of frequency, there are imbalances which are *(1)* invariably lethal, *(2)* almost always lethal, *(3)* usually lethal, and—the least frequent category—*(4)* usually survivable. These risk figures are likely to be valid irrespective of the mode of ascertainment of the family or of the identity of the other chromosome contributing the telomeric tip, at least in the majority of translocations.

By way of example, imagine that a carrier in the t(4;12)(p14;p13) family of Mortimer et al. (1980) noted earlier had sought advice about their own risk to have an abnormal baby. The single-segment involved is 4p14-pter. According to the rules set out earlier, adjacent-1 segregation is the category that implies risk for viable imbalance in this family translocation. Consulting Table 5–4, therefore, we see that the risk for imbalance (whether deletion or duplication) is given as 15.4%. The standard deviation (± 4.5) is quite small, indicating that the estimate is based on a good number of cases. But we also pay attention to the datum “+ 7.7” with reference to unkaryotyped stillbirths and neonatal deaths, many of which will have been, surely, chromosomally abnormal (probably Wolf-Hirschhorn syndrome). So the true figure to have an abnormal baby at term, who might or might not live, could well be 15.4 + 7.7 = 23.1%. A “private estimate” in this family had come up with a figure of 25%, which is sufficiently close to 23.1% to provide reassurance as to its accuracy.

Adjacent-1 Segregation, Double Segment

Every double-segment translocation is likely to be a unique case (or at least no other described family is known), and risk assessment is less precise. One known recurrent double-segment translocation, the t(4;8)(p16;p23), has been seen in sufficient numbers for a useful risk estimate to be derived (Table 5–5). Of course, if the family is large enough, a private segregation analysis will provide the best estimate; and eight other examples are listed in Table 5–5. Otherwise, Stengel-Rutkowski et al. (1988) recommend considering each segment separately. They propose the rule of thumb that the risk will be half that of the smaller of the two risk figures. Even this may be an overestimate; in many cases, the duplication/deficiency from a double-segment imbalance will be invariably lethal in utero—a risk of 0%—notwithstanding that each segment separately is on record with viability in the single-segment state. In one instance, however, this rule was vindicated, in the t(4;9)(p15.2;p13) family listed in Table 5–5. The risk attaching to 9p13, as a single-segment, is 6.4% (from Table 5–4), and this halved to 3.2%. From an actual family study, the comparable figure was also 3.2% (although a wide standard deviation of ± 3.2) (Midro et al., 2000).

Preimplantation genetic diagnosis (PGD) requires a different viewpoint, since in utero lethality has not had the chance to operate, and the denominator of the risk figure is quite different: this now refers to the rate of abnormalities in the day 3 embryo. In a double-segment translocation t(3;11)(q27.3;q24.3) carried by a brother and sister reported in Coonen et al. (2000), at least 15 out of 18 embryos of the brother were karyotypically unbalanced, and only one was normal or balanced. This one embryo was transferred, amniocentesis showed 46,XX,t(3;11), and a healthy carrier daughter was in due course born. His sister, a carrier of the same translocation, underwent two treatment cycles, with two out of six embryos apparently normal, but neither transferred successfully.

Adjacent-1 Segregation with Subtelomeric Double-Segment Exchange

The viable outcomes will be due to one or possibly both of the dup/del and del/dup combinations, and the family history may well be informative, as illustrated by the t(1q;3p) family reported in Kozma et al. (2004). Either of the combinations could arise from adjacent-1 segregation, or from independent 1:1 segregation of each normal homolog and its derivative chromosome (see Fig. **5–8**). From random 1:1 segregation, if that occurred, a theoretical risk of 50% for unbalanced karyotypes would apply at conception. More such cases will require study before a clearer picture can be drawn; but in any event a high risk, and as judged from the family history, is very probable.

In one PGD case reported, relating to a couple one of whom carried a t(2;17)(subqter;subqter), 13 of 18 embryos showed 2:2 segregation for the translocation (six alternate, seven adjacent-1), consistent with either two independent 1:1 events, or 2:2 disjunction from a quadrivalent (McKenzie et al., 2003). But the fact that the remaining five malsegregants displayed 3:1 disjunction suggests that a quadrivalent may indeed have formed, even if, considering the nature of this translocation, 3:1 is contrary to the “rules”

of malsegregation set out earlier.

Adjacent-2 Segregation

Very few translocations are capable of producing viable adjacent-2 segregant products, and the data on specific risk levels are limited (Table 5–6). Where the potential imbalance has considerable viability, for example, trisomy 9p and trisomy 21q, the risk is likely to be substantial and may be in the range of 20%–30%. The carrier mother in Figure **5–9** would have, from Table 5–4, an 18% risk for the recurrence of trisomy 9p.

3:1 Segregation, Tertiary Aneuploidy

In contrast to 2:2 segregation, the probabilities for unbalanced 3:1 outcomes differ between the sexes, with the female having the greater risk. For translocations other than the common t(11;22)(q23;q11), the risk is generally small and is less than 2%. Nevertheless, each translocation is entitled to its individuality, and atypically higher risks are possible, as may be exemplified in the t(12;13) noted earlier and shown in Figure **5–12**, in which two out of four children had a tertiary monosomy. In this case, it could be that the tiny derivative segregated independently, at random.

The common t(11;22) 3:1 segregation with tertiary trisomy is practically the only segregation mode to produce a viable abnormal baby in the common t(11;22)(q23;q11) (Fig. **5–11**). Different figures have been proposed for the level of risk. From the data of Stengel-Rutkowski et al. (1988), as listed in Table 5–6, the risk is 3.7% and <0.7%, respectively, for the female and male carrier. In a very large collaboration, with data from 110 families seen in 15 countries (there being some overlap with the material in Stengel-Rutkowski et al.), Iselius et al. (1983) arrived at risk figures for the female and male heterozygote, respectively, of 2.1% and 1.8%. Notably, in most of these families the index case was the only one known definitely to have the unbalanced karyotype. However, it could be supposed that reported malformed stillborn infants in these families were rather likely also to have had the unbalanced karyotype, and if this assumption is accepted, the risk figures for a live- or stillborn affected infant would increase to 5.7% and 5%, respectively. A rather higher risk figure for the female carrier, namely, ~10%, is due to Zackai and Emanuel (1980). These authors also observed that the chance of transmitting the translocation in balanced state is significantly greater than the theoretical 50%, with a risk of >70% in the families studied. As a further untoward consequence of being a carrier of this common t(11;22), the female may be at risk for breast cancer (Jobanputra et al., 2005; Wieland et al., 2006).

3:1 Segregation, Interchange Aneuploidy

The risk to have a child with Patau, Edwards, or Down syndrome from an interchange trisomy is remarkably small. It may be in the vicinity of 0.5% in the female, and less than this in the male (Stengel-Rutkowski et al., 1988). Upper limits of the estimated risks are given in Table 5–7. The figures for PGD can be much higher, as shown in Table 5–2, and as illustrated by the case of Conn et al. (1999) noted earlier, in which a woman with the karyotype 46,XX,t(6;21)(q13;q22.3) had 9/9 embryos with chromosome imbalance, including two with interchange trisomy 21, and one with probable interchange monosomy 6.

More than One Unbalanced Segregant Type

It is probably prudent to assume that where more than one mode of segregation can lead to a viable outcome, the overall risk will be cumulative and will be given by the sum of the individual risks. Thus, the carrier mother of the t(11;18)(p15;q11) shown in Figure **5–14** would have a risk comprising three components: duplication 18q11-qter due to adjacent-1; tertiary trisomy 18pter-q11 due to 3:1; and trisomy 18 due to 3:1 interchange. From Tables 5–4 and 5–7, and choosing the closest listed segments, these risks are 2.5%, <1.3%, and <0.2, respectively, for a total of up to 4.0%.[10]

Imprintable Chromosomes and Uniparental Disomy

Any translocation, of which a participating chromosome has an imprintable segment, is to be considered from this specific perspective. Engel and Antonarakis (2002) list 10 familial translocations from the literature associated with the birth of a child with a UPD syndrome, including Prader-Willi and Angelman syndromes (PWS and AS), Beckwith-Wiedemann syndrome, and UPD14; and Silver-Russell syndrome due to UPD7 has been reported in association with a maternal t(7;16) (Dupont et al., 2002). The gender of the transmitting parent becomes of relevance: for example, in a family segregating a rcp(4;11)(q35;p15.5), the unbalanced state 46,der(4) was expressed differently in children inheriting the der(4) from father or mother (Bliek et al., 2009). Overgrowth was part of the phenotype with paternal transmission, and growth restriction with inheritance from the mother, reflecting the different imprinted status of the 11p15.5 translocated segment on the der(4). The most notable phenotypic distinction is seen in differential parental transmission of 15q13 imbalances, leading to either PWS or AS.

A potential risk for UPD following postzygotic “correction” was noted earlier. What looks like alternate segregation in the fetus could actually have been 3:1 interchange trisomy, with a postconceptual loss of the homolog in question. In practice, this appears to be an exceedingly rare outcome (Dupont et al., 2002; Kotzot, 2008a; Heidemann et al., 2010). An example is the case in Calounova et al. (2006): a child with PWS had the same 46,XX,t(8;15)(q24.1;q21.2) karyotype as her mother, with absence of a paternal chromosome 15 and thus with UPD15mat. Another mechanism, rare indeed, is gametic complementation (see later discussion). This much is certain: any translocation involving chromosome 15 in particular is to be approached very circumspectly. The translocation with a 15q11–13 breakpoint is a special case, and unequal meiotic crossing-over can give a recombinant chromosome cytogenetically indistinguishable from the balanced state (Horsthemke et al., 1996).

Phenotype and Survivability

A major degree of dysmorphogenesis, involving several body systems, and globally disordered brain function is the typical picture in viable autosomal imbalance. Many patients will come with the knowledge of the particular phenotype of at least one of the viable segregant outcomes—the proband in their own family. The same imbalance in a future pregnancy would be expected to lead to a similar physical and mental phenotype.[11] Survivability is less predictable because, for many conditions, there is a fine line between relative robustness and a fragile hold on existence, intrapartum and postnatally. Whether there is a heart defect (a frequent malformation in many chromosomal disorders) may be a major factor in this. As for the phenotype of potentially survivable outcomes other than those already exemplified in the family, reference to the chromosomal catalogs and databases (de Grouchy and Turleau; Schinzel; ECARUCA; UKCAD) and to the journal literature provides a guide. For imprintable chromosomes, there may be an influence of the parental origin of the aneuploid segment, as noted earlier.

The Parental Balanced Translocation in the Fetus

The conventional wisdom is that if the same (balanced) karyotype found in the carrier parent is detected at prenatal diagnosis, there is no increased risk for phenotypic abnormality in the child: like parent, like child. Some have doubted this, and Fryns et al. (1992a) measured a 6.4% risk of mental and/or physical defects in the heterozygous children of translocation carriers (this figure including the background risk of 2%–3%). Others remain sceptical and impute ascertainment bias as the confounding factor (Steinbach, 1986). Theoretical mechanisms whereby an apparently balanced translocation could have a deleterious consequence, the parental normality notwithstanding, include the following four: a cryptic unbalanced defect beyond the resolution of routine cytogenetics (but nowadays possibly detectable on microarray); the postzygotic loss of a derivative chromosome in one cell line, converting an unbalanced to a mosaic balanced/unbalanced state; a position effect; and uniparental disomy.

Concerning the cryptic unbalanced defect, Wagstaff and Hemann (1995) provide a disconcerting example: an apparently balanced parental reciprocal translocation which turned out to be a complex chromosome rearrangement, with a tiny segment from the breakpoint of one of the translocation chromosomes inserted into a third chromosome (p. 216 and Fig. 12–7 in Chapters 12). In families in which the balanced translocation has been transmitted to numerous phenotypically normal individuals, such a scenario is most unlikely, since consistent co-segregation of the “cryptic chromosome” to give an overall balanced complement in all these persons would be improbable. Where the translocation is of more recent origin, perhaps de novo in the parent, the possibility may be more real. As mentioned earlier, the increasing sophistication of molecular

cytogenetics may enable such cases to be teased out. Clearly, if this sort of investigation is to be done, it should be well ahead of any pregnancy.

The case reported by Dufke et al. (2001) illustrates the possible scenario of mosaicism. An abnormal child with the same balanced t(17;22)(q24.2;q11.23) as his mother on peripheral blood analysis, showed, on skin fibroblast culture, a 47,t(17;22),+der(22) karyotype. This mosaic picture may reflect there having been a "interchange tertiary trisomy"[7] complement in the conceptus, with postzygotic loss of one of the two der(22)s in blood-forming tissue. A similar scenario is documented in Prontera et al. (2006): a mother carrying a t(1;15)(q10;p11) had an abnormal child, in whom the same apparently balanced karyotype had been shown at prenatal diagnosis. In view of the abnormal phenotype, a stringent postnatal analysis was done, which revealed a small fraction of cells, 4% (on blood), with trisomy 15; the conclusion is thus drawn that the initial conception had been from a 3:1 malsegregation with interchange trisomy, and a mitotic "correction" thereafter resulted in loss of the additional chromosome 15 in a substantial fraction of cells, but obviously not all. These reports raise the question: could the excess noted by Fryns et al. in a postnatal population be accounted for, in part at least, by this process? And, if so, could this be the basis of a misleading prenatal diagnosis? In fact, it could be imagined that, if the mother in Dufke et al. had had an amniocentesis, the unbalanced 47,t(17;22),+der(22) state would have been seen, since the sampling of amniocytes is somewhat equivalent to taking several skin biopsies. On that premise, it could be argued that a good number of normal cells/colonies from amniocentesis might indicate an unlikelihood of any such mosaicism, albeit that the very low degree of mosaicism in the other case described earlier would argue against complete reassurance in this respect.

The concept of "position effect" was discussed earlier. A particular gene in the close vicinity of a translocation breakpoint may function normally in a parent; but, for whatever reason, in the child the gene in question may be silenced, due to an effect of the adjacent chromatin of the other participating chromosome. Realistically, however, a question of position effect is worth raising only in families where the abnormal phenotype has already occurred. Of similar extreme rarity, there is the theoretical question of uniparental disomy following postzygotic "correction" of an interchange trisomy for an imprintable chromosome.

If an important additional risk due to one or other of the aforementioned scenarios really does exist, it is surely very small, perhaps no more than "a fraction of a percent" that a child with the "balanced" parental karyotype might have a defect of mostly unpredictable severity and extent. In the meantime, it remains true that in the great majority the balanced translocation really is balanced, structurally and functionally, and will have, of itself, no detrimental effect. Thus, in practical terms, it would be appropriate to advise continuing a pregnancy when the fetal karyotype is the same as that of the carrier parent, and with very considerable (if not absolute) confidence of a normal outcome.

Infertility and Pregnancy Loss

Infertility

Occasionally, some male translocation carriers are infertile with a spermatogenic arrest, as discussed earlier. There is little (if any) increased incidence of infertility in the female; from this point of view, oögenesis is a more robust process.

Miscarriage

Conceptions with large imbalances will abort. Against the background population risk of 15% for a recognized pregnancy to miscarry,[12] the risk for the translocation carrier is rather greater and is in the range of 20%–30% (Stengel-Rutkowski et al., 1988). For a few, the risk is very high, well over 50%. An increasing viability of conceptuses implies a correspondingly diminishing likelihood of pregnancy loss by miscarriage. Not to diminish the distress felt at the loss of a welcomed and wanted pregnancy, patients can be heartened that miscarriage, in this setting, is the natural elimination of a severe abnormality, which provides the opportunity to make a fresh, and hopefully, a more fortunate start. For a couple having lost all pregnancies to miscarriage, karyotyping in the previous generation may be helpful. The consultand would, in him or her self, embody the proof that the heterozygote *can* have a normal child, should one of his or her parents also be a carrier. Optimism has to be muted, however, in the setting of a family history of many miscarriages, which may indicate a propensity for the production of unbalanced gametes.

Preimplantation Genetic Diagnosis.

PGD has obvious attraction as a means to avoid a pregnancy with an imbalance, by choosing only embryos with a balanced complement, following embryo biopsy. Indeed, the practical observation is that this approach may substantially improve the chance for a translocation carrier to have a normal child, especially in those who have had several miscarriages and no liveborn child (Otani et al., 2006; and see Chapters 26). As we have commented a number of times previously, the risk figures in this context will be rather different. A chromosomally abnormal embryo at day 3 (when PGD is applied) has not been subject to selection pressure, and so a wide range of imbalances may be seen, the very great majority of which could never survive to term, and many of which would fail even before implantation (day 5). The data in Table 5–2 might suggest a 43% chance for a normal/balanced embryo from a male heterozygote, and 50% from a female. But the outlook may be less promising than this, as more data come to hand; and the average chance to obtain a normal/balanced embryo may be as low as 20% (Goosens et al., 2009). Obviously, couples in this situation will hope that their in vitro fertilization (IVF) team can produce a good number of embryos.

Other Issues

Other Family Members with the Same Translocation.

It appears to be the case that a translocation studied in one family member will typically display similar meiotic behavior in other carriers in the family; at least, this applies to the male, in whom gametic analysis is more readily pursued (Benet et al., 2005; Wiland et al., 2007). Thus, genetic advice can be, in practice, the same, for one and all.

Associated Mendelian Condition.

Rare translocations are associated with a mendelian disorder due either to the breakpoint disrupting or influencing a locus, or with coincidental linkage to a mutation near the breakpoint. We note some examples in the earlier section on "Biology." In such families, over and above any risk associated with unbalanced segregants, one should discuss the risk of transmitting the abnormality peculiar to that chromosome.

Cancer Risk.

In rare familial translocations, the rearrangement may promote mitotic malsegregation, or disrupt a tumor suppressor gene, and thus comprise a "hit" in the cascade of events leading to the cellular phenotype of cancer. A well-recognized case is that of chromosome 3 translocations implicated in familial renal cancer, of which a number of examples have been published (Valle et al., 2005). According to one construction, a three-hit sequence is envisaged, the first hit being the actual inheritance of the balanced translocation. Then, the mechanism is a mitotic malsegregation in an embryonal kidney cell. The derivative chromosome containing the 3p segment is lost, and in consequence one daughter cell, and thus the lineage from it, has only one copy of distal 3p, on the normal homolog (the second hit). Thereafter, on this remaining normal chromosome, a somatic mutation occurs in postnatal life at a 3p-located tumor suppressor gene in a kidney cell within this lineage (the third hit); and now the stage is set for a renal cancer to come into being.

Otherwise, a t(3;6) is recorded which possibly predisposes to hematological malignancy (Markkanen et al., 1987). Two families are known with familial adenomatous polyposis being due to a constitutional reciprocal translocation, t(5;10)(q22;q25) and t(5;8)(q22;p23.1), the relevant tumor suppressor gene (*APC*) having been disrupted at the 5q22 breakpoint (van der Luijt et al., 1995; Koorey et al., 2000). A father and daughter with an apparently balanced t(1;22)(p36.1;q12) both had neurofibromatosis type 2, due to the *NF2* gene having been disrupted (Tsilchorozidou et al., 2004). Laureys et al. (1990) reported a child with a de novo t(1;17)(p36.2;q12) presenting with neuroblastoma; it may be that somatic loss of the der(17) and duplication of the normal chromosome 17 in cells of the nervous system set the stage for the tumor to arise (Van Roy et al., 2002). An excess of constitutional rearrangements in a series of children with various tumors suggests the possibility of a causative role for some of them (Betts et al., 2001). The possible increase in risk for breast cancer in the common t(11q;22q) has been noted previously. Where the cytogenetic-cancer associations are firm, heterozygotes should

receive appropriate counseling, and entry into a cancer surveillance program is appropriate. Often, the associations appear to be no more than fortuitous (given that rearrangements are not uncommon, and cancer is very common).

Interchromosomal Effect.

There had originally been concern that a reciprocal translocation heterozygote might be prone to produce gametes aneuploid for a chromosome not involved in the translocation, specifically, in this context, chromosomes 13, 18, 21, or X, and there has been the occasional report of a translocation carrier having offspring with chromosomal imbalance not related to the family's translocation (Couzin et al., 1987). Warburton (1985) reviewed the associations of reciprocal translocations and trisomy 21 from unbiased (amniocentesis) data and found no evidence to support the contention. Uchida and Freeman (1986) and Schinzel et al. (1992) studied families in which a child with trisomy 21 also had a balanced translocation, and while in several the translocation was of paternal origin, in fact the extra chromosome 21 came from the mother.

More directly, numerous sperm karyotyping studies have, for the most part, shown no increase in disomies unrelated to the translocation, although some workers have raised doubts (Estop et al., 2000; Oliver-Bonet et al., 2004; Machev et al., 2005). Pellestor et al. (2001) suggest that carrier males with poor semen indices are the only ones in whom any such effect might exist; in which case, it might be the altered testicular environment, rather than the translocation of itself, that is the cause (Kirkpatrick et al., 2008). Analysis of embryos at preimplantation diagnosis supports the case against any effect (Gianaroli et al., 2002). Possibly, some specific translocations may have a very small individual risk, but there seems little reason to withdraw from the generality of Jacobs' assessment from 1979, and in practical terms we expect this view to prevail: "there is no indication that parents with a structural abnormality are at an increased risk of producing a child with a chromosomal abnormality independent of the parental rearrangement … [and] their recurrence risk for such an event is the same as the incidence rate in the population." Only with infertile men (needing ICSI for conception) might there really be a small risk, and as noted earlier, this may be due more to the infertility per se. No doubt the debate will continue.

Notes:

[1] There is scope for confusion in the use of these terms: of course, all reciprocal exchanges, by definition, involve two segments. A true single-segment exchange—that is, a one-way translocation—is generally considered not to exist, in that a segment of chromosome cannot attach to an intact telomere, although there are rare exceptions to this rule. The distinction begins to break down when a translocated segment is very small but could still contain genes (as in the subtelomeric translocation). Be this as it may, the terms double- and single-segment exchange, used knowledgeably, serve a practical purpose.

[2] Not "alternatively," as some publications erroneously use.

[3] The reader wishing to study further worked examples is referred to Midro et al. (1992), who analyze in some detail a series of translocations of differing risk potentials.

[4] This same 4;8 translocation has been observed in a small number of unrelated families, and it may be, after the t(11;22) noted below, the most frequent human reciprocal translocation. This recurrence reflects the presence in distal 4p and 8p of "olfactory-receptor clusters," which can act as recombination-predisposing duplicons (Maas et al., 2007). Other recurrent rearrangements are the translocations t(4;18)(q35;q23) and t(8;22)(q24.13;q11.21) (Horbinski et al., 2008; Sheridan et al., 2010). Some apparent recurrences may actually reflect unrecognized identity by descent (Youings et al., 2004).

[5] This is not official ISCN nomenclature.

[6] One breakpoint may compromise the function of a tumor suppressor gene; there is preliminary evidence that the t(11;22) carrier has an increased risk for breast cancer (Lindblom et al. 1994), but a definitive study, looking at large numbers in several kindreds, has yet to be done.

[7] Except in the extraordinary setting of postzygotic rescue. Kulharya et al. (2002) report a t(11;22) carrier mother having had a child from presumed adjacent-1 segregation with 46,XY,der(22) at conception, and then mitotic loss of the der(22) in one cell and duplication of the normal 22, leading to 46,XY,der(22)/46,XY mosaicism.

[8] Another route to this observation could be a 3:1 disjunction following a crossover in an interstitial segment (Petković et al., 1996).

[9] The ability to recognize a musical note—"perfect pitch"—is surely due to a brain gene, but this may be an asset, rather than a handicap; 7p22 may be the site of this genetic effect, with three unrelated musically gifted Danish translocation heterozygotes sharing this breakpoint (Bache et al., 2006).

[10] Intuitively, this figure may seem too low, given the well-known viability of partial and complete trisomy 18. The figure of 15% from HC Forum would seem to be more plausible.

[11] Similar may only mean "somewhat the same." Just as trisomy 21 presents a considerable range in intellectual capacity, variation may be observed with the identical segment, duplicated or deleted, in different family members. The rest of the (balanced) genome, which will of course differ, may dictate a relative vulnerability, or resistance, to the damaging effects of the imbalance.

[12] This figure applies respect to clinically diagnosable miscarriage, mostly occurring in the period 8 to 16 weeks gestation. Severely imbalanced forms may be lost as very early, even occult, abortions (p. 382).







Oxford Medicine







Chromosome Abnormalities and Genetic Counseling (4 ed.)

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Sex Chromosome Translocations

Chapter: Sex Chromosome Translocations
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THE SEX CHROMOSOMES (gonosomes) are different, and sex chromosome translocations need to be considered separately from translocations between autosomes. A sex chromosome can engage in translocation with an autosome, with the other sex chromosome, or even with its homolog. The unique qualities of the sex chromosomes have unique implications in terms of the genetic functioning of gonosome-autosome translocations. Unlike any other chromosome, the X chromosome is capable of undergoing "transcriptional silencing" or, as more usually spoken, facultative inactivation, of almost its entire genetic content. This fact has crucial consequences for those who carry an X-autosome translocation, in both the balanced and the unbalanced states. And unlike any other chromosome, the Y is composed of chromatin which is, in large part, permanently inert. Some translocations of this inert material can thus be of no clinical significance.

Biology

The X-Autosome Translocation

Both females and males can carry, as heterozygotes or hemizygotes, an X-autosome translocation, in balanced or unbalanced state. But the implications for the two sexes are rather different, and we therefore need to treat the two cases separately. First, we need to review the concept of X-inactivation.

X-Inactivation

The normal female has two X chromosomes, and yet the possession of only a single X is sufficient to produce normality in the 46,XY male. Are the sexes really so genetically different? Does the female really need a second X? The answer is a qualified no. The second X is largely surplus to requirement, and it is subject to transcriptional silencing. Very early in embryonic existence, around the period of the morula and blastocyst stages, a process is initiated whereby one of the X chromosomes in every cell of the female conceptus is randomly genetically inactivated (van den Berg et al., 2009). This process is called (after Dr. Mary Lyon) *lyonization*. In all descendant progeny cells thereafter, the same X chromosomes remain inactive or active, respectively. This "dosage compensation" allows for a functional monosomy of most of the X chromosome.

Transcriptional silencing is initiated at an X-inactivation center (XIC) in Xq13 (Fig. **6–1**), and it spreads in both directions along the chromosome. Within the XIC is a gene *XIST* that is cis-acting (that is, it can influence only the chromosome that it is actually on), and that is transcribed only from the *in*activated X. This transcript, named "XIST," for X (inactive) specific transcript, is not translated into protein but functions as an RNA molecule. The XIST RNA "coats" the X chromatin and may act first by influencing the degree of acetylation and other modification of the histones, and this then preventing the DNA from being transcribed. This inactive state is then "locked in" by methylation of CpG islands, and this methylation status remains in place in the descendant daughter cells. The reader wishing full detail is referred to Migeon's *Females Are Mosaics* (2007). Normal women can have quite skewed ratios of active X^m and inactive X^p chromosomes[1] and vice versa, even more than 90:10, and there can be differences in ratios between different tissues in a woman (Sharp et al., 2000). The inactive X replicates late during the cell cycle; the active X replicates early, along with the autosomes.

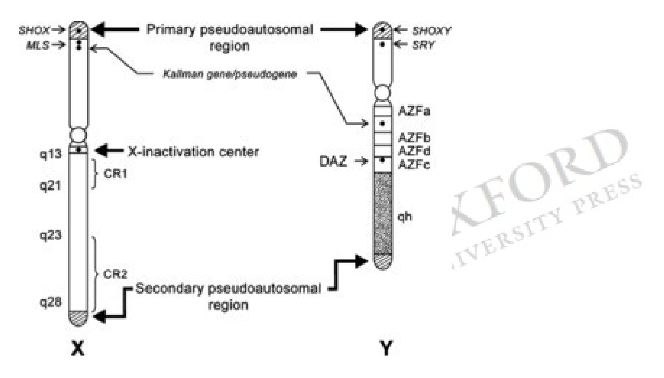




Figure 6–1
Notable regions of the sex chromosomes. AZF, azoöspermia factor regions a–d. Dots show specific loci: *DAZ*, deleted in azoöspermia locus; *MLS*, microöphthalmia with linear

skin lesion gene; *SHOX*, short stature homeobox gene (X chromosome), *SHOXY*, short stature homeobox gene (Y chromosome); *SRY*, testis-determining locus. CR1, 2 show critical regions 1 and 2 (p. 116).

But this is not to say that the female's second X chromosome is unnecessary (a rather obvious statement, considering the difference between 46,XX and 45,X individuals). Not all genes on the X chromosome are inactivated, and thus some loci are, in the normal female, functionally disomic. There is a block to the spread of inactivation into the primary pseudoautosomal region (PAR1), which comprises the terminal 2.6 megabases of Xp in band p22.3; this segment has an homologous region on distal Yp (Fig. **6–1**). There is a secondary PAR (PAR2), which extends over 320 kb within distal Xq, having homology with distal Yq (Kvaløy et al., 1994). An obligate recombination event occurs in the PAR1 of the X and the Y chromosome at male meiosis; recombination between the secondary PARs, if it occurs at all, is infrequent. Certain other loci elsewhere on the X than in the PARs (some of which have homologs on the Y) are not subject to inactivation, and disomic expression of these genes in the female (and, for some, in the male) is normal (Disteche, 1995). One such is the nonpseudoautosomal X-Y homologous region, at Xq21.3 and Yp11.2, respectively, and in fact this is the largest region, some 4 Mb in length, of shared sequence between the sex chromosomes (Wilson et al., 2007).

The Female X-Autosomal Heterozygote

The balanced X-autosome translocation carrier has two translocation chromosomes, the der(X) and the der(autosome). The X segment in one of these, most commonly the der(X), contains the XIC, and the X segment in the other, usually on the der(autosome), lacks the XIC. The latter segment, having no XIC of its own and being beyond the influence of the XIC on the other derivative, is always active. The only way, then, for the karyotypically balanced female X-autosome heterozygote to achieve a functionally balanced genome is to use, as her active X complement, the two parts of the X in the two translocation chromosomes: together, they add up to an equivalent whole, and functioning, X chromosome. The other chromosome, the *normal* X, is inactive. The cartoon karyotype in the 46,X, t(X;12) carrier mother in Figure **6–2** shows the normal X as inactive (dotted outline), and the X-segments of the der(X) and der(12) as active (solid outline).

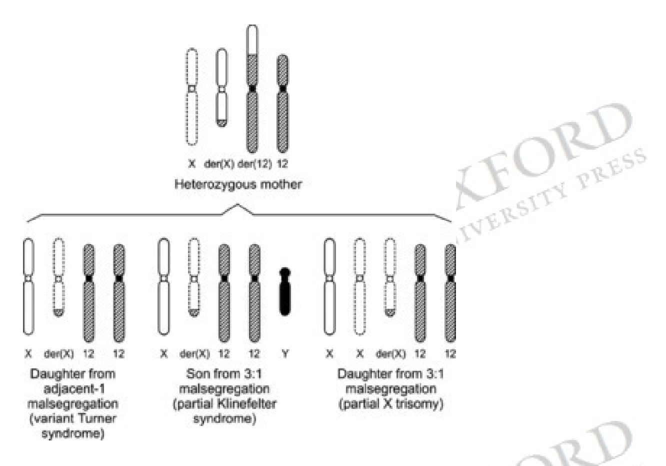


Figure 6–2
Inactivation patterns. Mother with a *balanced* X-autosome translocation, showing patterns of inactivation in herself and in her two chromosomally *unbalanced* children with partial Turner and partial Klinefelter syndrome, respectively. Dashed outline indicates inactivated chromosome. The inactivation pattern of a theoretical third child with a partial X trisomy is shown at right. Note that the balanced carrier inactivates her normal X chromosome, while it is the abnormal X which is inactivated in the unbalanced offspring (and, in the third child, one of the additional normal X chromosomes as well). Based on family in Figure **6–4**.

Probably, the mechanism to bring about this asymmetric inactivation is as follows. Inactivation is initiated at random in each cell, at either one of the XICs. Some cells will be functionally balanced, with the intact X inactive, as described earlier. Others, in which the intact X is active, will have a functional disomy for the X chromosome segment that is translocated to the der(autosome), due to this X segment not being subject to transcriptional silencing, and thus genetically active. According to this theory, cell selection then eliminates the functionally partially disomic X lines (Fig. **6–3**, sequence a→b→c). This mechanism is successful in a fraction of translocation heterozygotes, and aside from a possible gonadal effect (see later), such individuals are phenotypically normal.

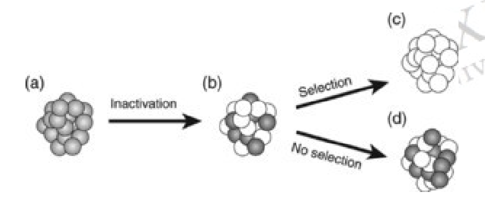


Figure 6–3
Skewing or nonskewing of X chromosome inactivation, as a theoretical explanation for the X-autosome carrier being of either normal or of abnormal phenotype (and see text). (*a*) Before X-inactivation occurs, both the normal and the der(X) are active in all cells (shown in light gray). (*b*) X-inactivation occurs as a random, cell-autonomous process. Cells shown in white have the der(X) as the active chromosome, and thus the genetic activity of these cells is balanced with respect to X chromosomal output. The cells shown in dark gray have the normal X-active, and in consequence their X chromosomal activity is imbalanced, due to the additional output from the X-segment of the der(autosome). Subsequently in embryonic development: *Either* (*c*) the cells with the normal X-active (dark gray) die out, due to their functional genetic imbalance, leaving only the cells with the der(X) active (white). These latter cells functionally are genetically balanced, and the phenotype is normal. The individual has a skewed X-inactivation pattern.[5] *Or* (*d*) the dark gray cells persist, in spite of their functional genetic imbalance (the defect is not severe enough to be lethal), and the individual is a mosaic of functionally balanced tissue (white cells) and imbalanced tissue (dark gray cells). In consequence, the phenotype is abnormal. (Adapted from Lanasa and Hogge, 2000).

This mechanism, as it would seem, may not infrequently fail, and phenotypic abnormality is the consequence. “Not infrequently” may translate to as much as 25%, with reference to the literature study of Schmidt and Du Sart (1992). If some functionally disomic cells survive and come to comprise part of the soma (Fig. **6–3**, sequence a→b→d), this would, presumably, have some deleterious effect. The natural prediction is that only cells with small partial disomies would be capable of survival. Thus, we might more commonly expect to observe, in these affected carrier females, translocation breakpoints in distal Xp or distal Xq (Xp22 and Xq28), which would impart disomy for only a very small segment of either distal X short arm or distal X long arm. This was indeed the observation in the reviews of Schmidt and Du Sart (1992) and Du Sart et al. (1992). To the contrary, however, the X breakpoints in the data assembled from a survey conducted by the U. K. Association of Clinical Cytogeneticists showed a wider

distribution, and the X and der(X) did not necessarily display a skewed inactivation (Waters et al., 2001; A. J. M. Crocker, personal communication, 2001). There was no particular predilection for site of breakpoint, other than Xq22 being overrepresented. These authors believe that their data may be more representative, having avoided a reporting bias. Given their observations, they propose that gene disruption may be similarly important as a factor causing phenotypic abnormality. Part of the difference between the two studies may lie in the nature of the presenting phenotypes, whether these be malformation/cognitive compromise, or merely compromise of ovarian function. More such data would be useful.

Measuring Inactivation Status.

Inactivation status can be assessed cytogenetically (replication-banding, or R-banding), which enables, in principle, distinction of the early replicating (active) and the late replicating (inactivated) X chromosomes, and allows a precise estimate of the ratio of normal-X^{active} to translocation-X^{active} cells. Mostly, however, the analysis is done using molecular methodologies. The androgen receptor locus, at Xq13 (quite close to the XIC), is often used as the basis of this test. In the phenotypically normal heterozygote, the observation of a complete skew of translocation-X^{active} and normal-X^{active}, in the representative tissue analyzed, would indicate that the same 100:0 proportion applied elsewhere in the soma. Since it is impossible ever to test the entire soma (and in particular the brain), it would have to remain an open question, in a phenotypically abnormal but structurally balanced X-autosome heterozygote, that a more random skewing pattern might apply in some tissues, notwithstanding a complete skew in the peripheral tissue(s) tested. Abnormal individuals may show incomplete intertissue concordance of inactivation status, with sometimes quite different ratios in different tissues: say, 80:20 in blood and 30:70 in skin (Schmidt and Du Sart, 1992).

Ovarian Function and The X “Critical Regions”

Breakpoints at certain locations in the X may affect ovarian function (Table 6–1). A breakage and reunion within either of two “critical regions” (CR1 and CR2[2]) is characteristically associated with premature ovarian failure. CR1 is located in Xq21.1, and CR2 in Xq23-q28 (Fig. **6–1**) (Rizzolio et al., 2006). In the case of Xq21 breakpoints, these may lead to an epigenetic down-regulation of “ovarian genes” located on the translocated autosomal segment, whereas breaks in Xq23-q28 may affect genes in the vicinity that normally require disomic expression (Rizzolio et al., 2009). In one series of 30 women presenting with premature ovarian failure, in whom the cytogenetic findings were reviewed, Devi and Benn (1999) recorded just one to be an X-autosome translocation heterozygote; thus, it is an infrequent cause of this problem.

Table 6–1. Occurrence of Gonadal Dysgenesis (Primary or Secondary) in 118 t(X-Autosome) Women According to X Chromosome Breakpoint

BREAKPOINT	GONADAL DYSGENESIS	NORMAL GONADAL FUNCTION
Xpter-q12	5	37
q13	4	8
q13-q22	20	1
q22	11	6
q22-q25	7	1
q26	3	5
q27-qter	1	9

Source: From Therman et al. (1990).

The Male X-Autosomal Hemizygote

Almost invariably, the cytogenetically balanced male hemizygote is, without intervention, infertile, due to spermatogenic arrest; disruption of the sex vesicle (see later discussion) is the presumed proximate cause of the obstruction (Hwang et al., 2007). In two men subject to testicular biopsy, Quack et al. (1988) showed germ cell maturation arrest mostly at the pachytene stage of meiosis I, although a few cells managed to make the first and some even the second meiotic metaphase, and thus might have become spermatozoa. This outcome of a very modest success might more likely be achieved in those men in whom the breakpoints are more centromerically placed. A man hemizygous for a whole-arm translocation (X;18)(q11;p11.1) was subject to sperm chromosomal fluorescence in situ hybridization (FISH) analysis; he had presented with infertility and “very severe oligoasthenoteratozoöspermia.” Analysis showed a range of segregant types in the small number of 447 cells able to be studied: alternate segregation in just over 50% (with half of these normal 23,Y), and adjacent-1, adjacent-2, 3:1, and 4:0 in 8%, 5%, 22%, and 2%, respectively (Perrin et al., 2008). If sperm can be retrieved, intracytoplasmic sperm injection (ICSI) may be attempted in the carrier male in order to enable fertility (see later).

Patterns of Inactivation in the Unbalanced Offspring

Female Offspring of the X-Autosomal Heterozygote or Hemizygote

As a rule (but one that can be broken), the pattern of inactivation that is observed, following selection, will be the one that allows the least amount of functional imbalance. This is typically arrived at in the karyotypically unbalanced daughter by inactivation of the *abnormal* chromosome, always supposing that the choice exists (and the choice can exist only if the abnormal chromosome contains an XIC).

If the abnormal chromosome is a der(X) from a single-segment exchange, containing no autosomal material other than a telomeric tip, it comprises, essentially, a deleted Xp or Xq chromosome. In a girl with the 46,X,der(X) karyotype, preferential inactivation of this deleted X leads simply to a phenotype of partial Turner syndrome. Consider the family segregating a t(X;12) shown in Figures **6–2** and **6–4**. The segregation shown in Figure **6–2** (daughter from adjacent-1) and Figure **6–4a** (daughter) illustrates the case for an Xq deletion. Here, the normal X is active (shown as solid outline in Fig. **6–2**), and the der(X) inactivated (dotted outline). Leichtman et al. (1978) provide an example of the Xp deletion circumstance in a three-generation family with seven persons having an Xp–Turner syndrome variant on the basis of a segregating t(X;1).

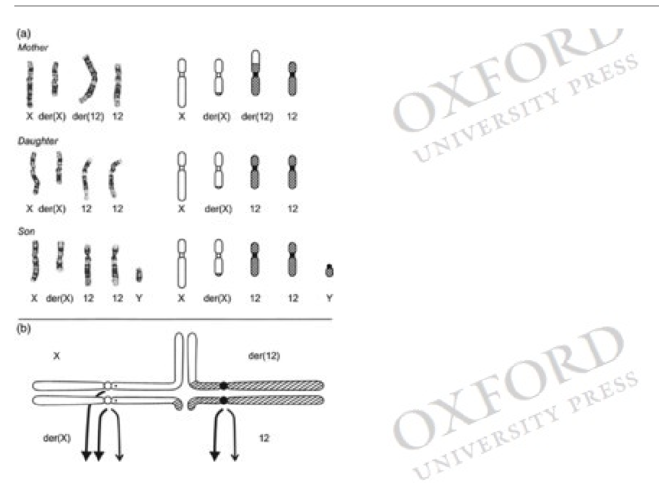




Figure 6–4
(*a*) Mother with balanced X;12 translocation, showing two different segregant outcomes. Her daughter had presented with clinical Turner syndrome, in whom the karyotype was initially interpreted as del(X)(q22). Her son was subsequently studied, and he had a partial Klinefelter syndrome. (Case of J. A. Sullivan.) (*b*) The presumed pachytene configuration during gametogenesis in the mother (X chromatin, open; chromosome 12 chromatin, crosshatched; dot indicates X-inactivation center). Light arrows indicate movements of chromosomes to daughter cells in adjacent-1 segregation, as observed in the daughter with partial Turner syndrome. Heavy arrows show the tertiary trisomy combination seen in the son with partial Klinefelter syndrome. These two segregations are represented in *b* and *c* in Figure **6–7**.

If the der(X) carries a larger translocated autosomal segment—conferring, therefore, a partial autosomal trisomy in the 46,X,der(X) subject—the effects of this imbalance may be mitigated by selective inactivation of the abnormal chromosome. Transcriptional silencing can spread, albeit patchily, into the autosomal chromatin on the der(X), converting, at least partially, a structural autosomal trisomy into a functional autosomal disomy. Consider these two cases in which a practically complete trisomy 15—which typically causes first-trimester abortion (Rajcan-Separovic et al., 2001)—produced, in comparison, a very much attenuated phenotype. Garcia-Heras et al. (1998) reported on the terminated pregnancy of a t(X;15)(p22.2;q11.2) carrier mother, from whom the 19-week fetus with the der(X) was trisomic for 15q11.2-qter, but only rather mild abnormalities of fetal morphology were to be noted. A t(X;15)(q22;q11.2) involving the same 15q11.2 breakpoint (in this case de novo) and diagnosed in a mildly dysmorphic and moderately developmentally delayed 3-year-old child, is described in Stankiewicz et al. (2006).

A number of similar cases are on record, and the degree to which a phenotype may be attenuated is presumably related to the extent that genes in the autosomal segment are inactivated. Giorda et al. (2008) analyzed cells from a girl with mild dysmorphology, arthritis, obesity, microcephaly, and mental and behavioral disability, who had the karyotype 46,X,der(X)t(X;5)(q22.1;q31)dn and was thus partially trisomic for the large segment 5q31–5qter. They were able to show that, of 17 interpretable genes tested in this translocated segment, 9 had been inactivated, while another 8 were active (as measured by methylation status). This inactivation did not “weaken” as it spread further into 5q31–5qter segment, and indeed the autosomal gene closest to the Xq-5q breakpoint remained active; thus, some autosomal genes were susceptible, and some were resistant, to the spreading influence from the XIST of the der(X). Figure **6–9** shows an example of blocked spread of inactivation into the autosomal (16p) segment of an X;16 translocation: observe the der(X) in the lower row, with pale (inactivated) long arm and dark (active) short arm.

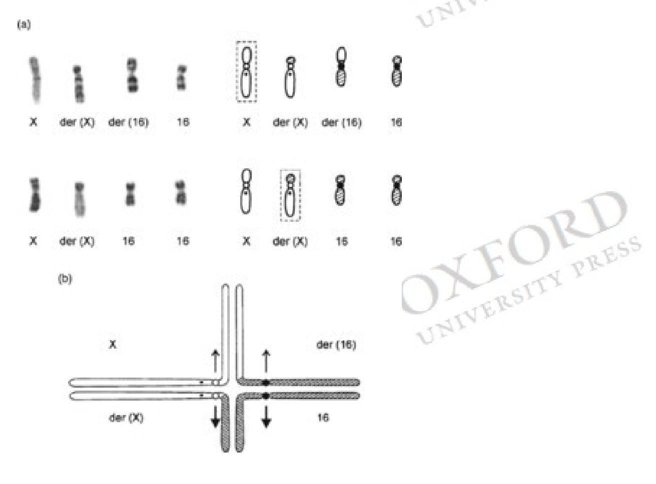




Figure 6–9
Spread of inactivation into autosomal segment. (*a*) Mother with balanced X;16 translocation (*above*), and her daughter with a 46,X,der(X) karyotype from adjacent-1 segregation (*below*). The translocation is t(X;16)(p11;p12). Replication-banding shows active (darker-staining) and inactive (lighter-staining) chromosome segments. The normal X is inactivated in all cells analyzed in the mother (dashed box on cartoon karyotype; dot indicates X-inactivation center). The daughter's abnormal X lacks Xp and contains distal 16p material. This chromosome is preferentially inactivated (dashed outline of box), but in 76% of cells analyzed (lymphocytes) the inactivation has not continued through the translocated 16p segment (dotted outline of box). The phenotype is the combined result of the Xp monosomy and a “partial” 16p trisomy. The child is short and has a developmental age of about 2½ at a chronological age of 4 years. (Case of C. E. Vaux.) One other daughter had the same balanced translocation as the mother and showed consistent inactivation of the normal X chromosome in blood lymphocytes, but she suffered intellectual deficit. (*b*) The presumed pachytene configuration during gametogenesis in the mother (X chromatin, open; chromosome 16 chromatin, crosshatched; dot indicates X-inactivation center). Arrows indicate movements of chromosomes to daughter cells in adjacent-1 segregation; heavy arrows show the combination observed in this family. This is essentially the segregation i in Figure **6–7**, with an Xp breakpoint in this case.

The converse, whereby the process of spreading autosomal inactivation may be detrimental to the phenotype, by converting a functional disomy (or near disomy) into a functional monosomy, is rarely observed. The family illustrated in Figure **6–11** provides a possible example. At first sight, one might have expected only a Turner syndrome phenotype in the daughter with a 45,X,der(X),–22 karyotype, since the essential defect appeared to comprise an Xp deletion, with her total complement of 22q material being intact, or nearly so. However, a more severe clinical picture evolved, and this may have reflected, speculatively, a transcriptional silencing of some crucial 22q loci, notwithstanding the apparent block to inactivation at the breakpoint on cytogenetic study. This case is mentioned further later.

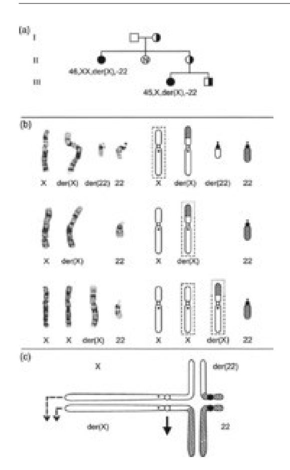






Figure 6–11
3:1 tertiary monosomy and adjacent-2 segregation both occurring in the same family (and see text). (*a*) Pedigree of family segregating a t(X;22)(p21.3;q11.21). Filled symbol, imbalanced state; half-filled symbols, heterozygote/hemizygote; N = 46,XX. (*b*) Partial karyotypes of heterozygotes (*top*), and of the two unbalanced states (*lower*). On replication-banding, the normal X is inactivated in all cells analyzed in the heterozygotes, whereas the der(X) is inactivated in the two affected persons (dashed box on cartoon karyotype; dot indicates X-inactivation center). In the affected child in generation III with a 45,X,–X,+der(X),–22 karyotype (middle karyotype), the der(X) was positive for a probe recognizing a sequence in the DiGeorge critical region. The der(X) chromosome showed, in 50/50 cells, apparently no inactivation going through to its 22 component (dotted outline of box), but the clinical picture might suggest otherwise (see text). The affected woman II:1 has the adjacent-2 karyotype 46,XX,+der(X),–22. (Case of T. Burgess.) (*c*) The presumed pachytene configuration during gametogenesis in the heterozygote (X chromatin, open; chromosome 22 chromatin, crosshatched). Heavy arrow indicates movement of the der(X) chromosome to one daughter cell in 3:1 segregation (essentially segregation *k*, Fig. **6–7**). Dashed arrows show the movement of chromosomes in the adjacent-2 combination (segregation *c*, Fig. **6–8**).

If, in the female with a 46,XX,der(autosome) karyotype, the derivative chromosome has no XIC in its translocated X-segment, this cannot be inactivated, and a functional partial X disomy is the consequence (Sivak et al., 1994). Figure **6–5** demonstrates a functional disomy for a part of Xp (Xp22.31-pter)[3] in a chromosomally unbalanced daughter; in this instance, since the autosomal breakpoint is at the telomere, we assume there to be little or no effect from a 10q monosomy. Gustashaw et al. (1994) describe a similar case in which they could be sure the Xp imbalance was the sole cause of the abnormal phenotype, since the autosomal breakpoint was in 13p and the loss of one acrocentric short arm has, of itself, no effect. Functional disomy of distal Xq, Xq28-qter, has been reported sufficiently often that a clear core phenotype can be described (Sanlaville et al., 2005).

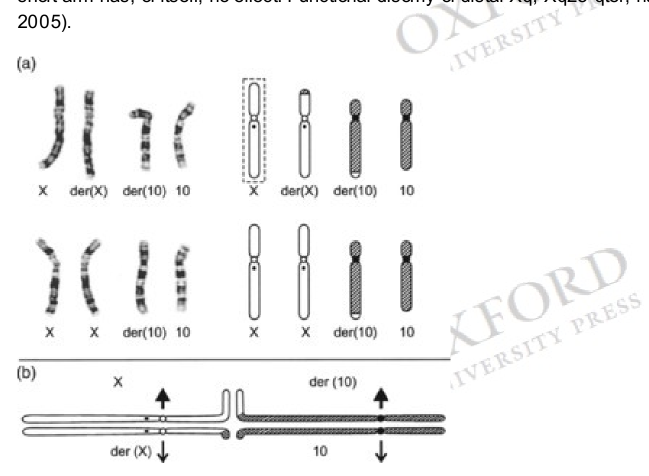




Figure 6–5
Functional X disomy. (*a*) Mother with balanced X;10 translocation (*above*), and her daughter with a 46,XX,der(10) karyotype from adjacent-1 segregation (*below*). The translocation is t(X;10)(p22.31;q26.3). Dashed box on cartoon karyotype indicates preferentially inactivated chromosome; dot indicates X-inactivation center. The der(10) contains Xp material in the translocated segment, which cannot be inactivated, and so the daughter has functional X disomy. Since the 10q breakpoint is in the terminal band we may regard this as an effectively single-segment exchange, with the phenotype of severe mental deficit and minor dysmorphism due entirely to disomy for the small Xp22.31→pter segment. (Case of A. Ma and H. R. Slater.) (*b*) The presumed pachytene configuration during gametogenesis in the mother (X chromatin, open; chromosome 10 chromatin, crosshatched; dot indicates X-inactivation center). Arrows indicate movements of chromosomes to daughter cells in adjacent-1 segregation; heavy arrows show the combination observed in this family. This is essentially the segregation *a* shown in Figure **6–7**.

Male Offspring of the Female X-Autosome Heterozygote

Analogous to the female, the male inheriting a der(autosome) is affected according to whether the X translocated segment does or does not contain an XIC. If the X-segment lacks an XIC, a functional X disomy ensues, with a severe phenotypic effect. If the X-segment contains an XIC, the X-segment is inactivated and, other things being equal, a Klinefelter-like phenotype might be expected. But this expectation might not be met, and a more severe clinical picture, whether due to incomplete inactivation, or to the effect of a concomitant autosomal deletion, could result. Balcı et al. (2007) report a three-generation family with a t(X;19)(q11;p13.3): a normal grandmother and mother with the balanced translocation, and a severely retarded boy, physically somewhat resembling Prader-Willi syndrome, whose karyotype was 46,XY,der(19)t(X;19). Virtually the entire Xq—including the XIC—was present in disomic dose on the der(19). Its otherwise lethal effect was considerably adjusted by inactivation, but nevertheless the phenotype was a great deal more severe than “Klinefelter-like.”

Origin of the X-Autosome Translocation.

All de novo balanced X-autosomal translocations so far studied have been of paternal origin, which may reflect the availability in male meiosis of the X chromosome for exchange with other chromosomes; the X pairs with the Y only at the PAR1, and the rest of the chromosome is unsynapsed (Turner, 2007). In one well-analyzed example,

Giacalone and Francke (1992) did a molecular dissection on a de novo t(X;4)(p21.2;q31.22) in a girl with Duchenne muscular dystrophy and proposed a format in which two GAAT sequences 5 kb apart in Xp, and one GAAT in 4q, came together during meiosis in spermatogenesis, deleted the 5 kb length in Xp (which comprised a small part of the *dystrophin* gene), and reformed as a der(X) and a der(4). Similar mechanisms likely underlie the formation of most X-autosome translocations. Once a balanced translocation is established in a family, and if the heterozygous state is associated with phenotypic normality, male infertility dictates that transmission thereafter will be matrilineal. We comment upon the de novo X-autosome translocation identified at prenatal diagnosis on p. 469.

Translocations That Disrupt X-Borne Loci.

Quite a number of X-linked disorders have been seen in females with (typically de novo) X-autosome translocations. The resulting clinical problems may manifest because of nonrandom X-inactivation, disruption of a gene, or a combination of both. With preferential inactivation of the normal X, there remains no functional copy of the normal allele: an effectively “nulliallelic” state. Among several other examples, we may point to the case of twin sisters with de novo balanced reciprocal translocations between Xp and 5q (Zenker et al., 2005). These girls had ornithine transcarbamylase (OTC) deficiency due to a combination of disruption of the OTC gene and skewed inactivation of the normal X chromosome. The disease state resulted, with no enzyme produced, because one allele was disrupted and the other allele was silenced. X-autosome translocations have offered research potential, and some loci have been initially mapped by study of rare/unique female patients with the particular mendelian condition (Schlessinger et al., 1993). The classic historical case is the female Xp21-autosome translocation heterozygote who has Duchenne/Becker muscular dystrophy, as mentioned earlier, and an example of which is illustrated in Figure **6–6**.

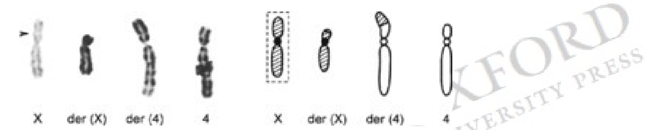


Figure 6–6
A de novo X-autosome translocation 46,X,t(X;4)(p21;p16) in which the *dystrophin* locus at the Xp21 breakpoint is presumed to be disrupted, in a 7-year-old girl. In consequence very little dystrophin is produced, and the girl has a Becker-like muscular dystrophy. The approximate position of the dystrophin locus is indicated (arrowhead) on the intact X. The intact X is preferentially inactivated, as shown here with replication-banding and indicated in dashed outline on the cartoon karyotype. Early replicating (active) chromatin and the late replicating (inactivated) chromatin stain dark and light, respectively. (Case of J. A. Sullivan.)

The X chromosome may have a particular load of “brain genes” (Turner, 1996), and it is of interest that a number of pure brain-related phenotypes, without dysmorphism or malformation, have been associated with these translocations. A girl with lissencephaly and an X;2 translocation pointed the way to the discovery of the *doublecortin* gene at Xq22.3 (Gleeson et al., 1998). The disruption of an X-linked neuronal gene, *oligophrenin-1*, caused isolated mental defect in a female with an X-autosome translocation 46,X,t(X;12)(q11;q15). The breakpoint was in the second intron, and thus the first two exons of the gene were on the der(12), and the remaining 23 exons on the der(X). No transcript could be produced, due to this disruption of the allele, and with the other allele on the normal X having been inactivated (Billuart et al., 1998). Another gene at Xq11, with effects in a number of compartments of the neural substrate (but not outside it), is *collybistin*, which influences a specific type of neuronal receptor. A woman with an X;18 translocation that disrupted *collybistin* (the breakpoint of the 18 was in a region devoid of genes) presented a syndrome of mental retardation, aggressive behavior, epilepsy, anxiety, and a disturbed sleep pattern (Kalscheuer et al., 2009). And rare patients with an X-linked dominant infantile spasm syndrome, reminiscent of the Rett syndrome phenotype, and having its basis in the gene *CDKL5*, have had translocations in which the X breakpoint is within the *CDKL5* locus at p22.1 (Córdova-Fletes et al., 2010).

Details of meiotic behavior

Female meiosis

In oögenesis, a quadrivalent presumably forms, just as in the two-way translocation between autosomes. 2:2 alternate segregation with the intact X and intact autosome can lead to 46,XX or 46,XY conceptions, while transmission of the translocation in balanced state produces heterozygous or hemizygous conceptions. As for malsegregation, Figures **6–7** and **6–8** set out certain outcomes that may be viable, for various categories of single-segment and double-segment translocation, as discussed later. Given the greater survivability of X imbalances due to inactivation, and likewise a possible lessened effect of autosomal imbalance, a greater number of conceptuses are potentially viable than from the autosome-autosome translocation. The “rules” of segregation (p. 77) may not apply; for example, a viable adjacent-2 malsegregation can occur with a derivative chromosome having a large centric segment. The coexistence of tertiary monosomy and adjacent-2 aneuploidy in the family described in Figure **6–11**, two otherwise very uncommon segregations, reflects the unique characteristics of the X-autosome translocation.

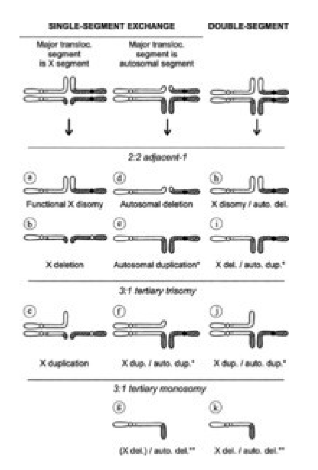






Figure 6–7
Major categories of adjacent-1 and 3:1 malsegregation in the X-autosome female carrier. The top row shows quadrivalents at maternal meiosis, and the following rows various combinations of segregant products. Open, X chromatin; crosshatched, autosomal chromatin; dot indicates X-inactivation center.

“Single-segment” and “double-segment” are defined in the text. X exchanges can occur in either Xp or Xq; only Xq exchanges shown here. Circled letters provide reference points for text comments.

*Effect of autosomal duplication may be lessened by spreading of transcriptional silencing into the autosomal segment of the der(X).

**Blocking of spread of inactivation into the autosomal segment of the der(X) may avoid further functional autosomal monosomic effect.

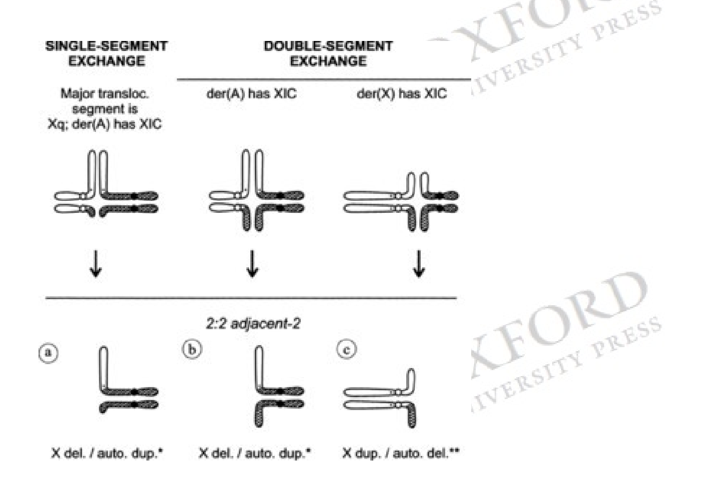




Figure 6–8
Three categories of adjacent-2 malsegregation in the X-autosome female carrier. The top row shows quadrivalents at maternal meiosis, and the next row various combinations of adjacent-2 segregant products. Note that these potentially viable outcomes occur only in the setting of the transmitted derivative chromosome, be it the der(X) or the der(autosome), having an X-inactivation center (XIC). In the first two columns, the der(autosome) has the XIC; here, the X breakpoint must be in proximal Xq, above the XIC, as depicted. In the third column, in which the der(X) has the XIC, X exchanges can occur either in Xp, or in Xq distal to the XIC; only an Xp exchange is shown here. Open, X chromatin; crosshatched, autosomal chromatin; dot indicates XIC; der(A), der(autosome). Circled letters provide reference points for text comments.

*Effect of autosomal duplication may be lessened by spreading of transcriptional silencing into the autosomal segment of the der(A).

**Blocking of spread of inactivation into the autosomal segment of the der(X) may avoid further functional autosomal monosomic effect.

Categories of Translocation and Modes of Malsegregation

We consider here various chromosomal scenarios, which ought to cover the majority of clinical circumstances. Concerning terminology with respect to the size of translocated segments: if one of the translocation breakpoints is at the telomeric tip of either the autosome or the X chromosome, and thus only one of the translocated segments (X or autosomal) comprises an important amount of chromatin, this may be considered an effective "single-segment exchange." If both translocated segments are of significant size, this is a "double-segment exchange."

Single-Segment Exchange, X Translocated Segment

The first two columns in Figure **6–7** and the first column in Figure **6–8**, segregations *a–c* and segregation *a*, respectively, depict the general form of a translocation in which the single important exchanged segment comprises X chromatin. A particular example is shown in Figure **6–4**, in which the derivative X chromosome is deleted for a large segment of Xq and has only the telomeric tip of 12p in exchange. A child receiving this abnormal "Xq–" in place of a normal X, or as an additional chromosome, could present with a partial form of a sex chromosome aneuploidy syndrome. Thus, a daughter with 46,X,der(X) from adjacent-1 malsegregation (*b* in Fig. **6–7**) would have a variant form of Turner syndrome. From tertiary trisomy (*c* in Fig. **6–7**), a son with 47,XY,+der(X) would have incomplete Klinefelter syndrome; and a 47,XX,+der(X) daughter might show the 47,XXX phenotype to a diminished degree.

More severe consequences follow the countertype adjacent-1 segregation, *a* in Figure **6–7**. Conceptions with 46,der(12) from adjacent-1 segregation would, in the family in Figure **6–4**, be functionally disomic for a large, unsurvivable amount of Xq, and they would abort. However, if the translocated X segment is small, the functionally disomic X state may be viable. This is shown in Figure **6–5**, in which the mentally retarded and dysmorphic daughter has a 46,XX,der(10) karyotype and is functionally disomic for the small amount of Xp22.31-pter.

As for adjacent-2 segregation (Fig. **6–8a**), such a gamete would, in theory, have viability only if it is the der(autosome) that is transmitted, along with the intact autosome, *and* if the X segment of the der(autosome) includes the XIC. In that case, inactivation could spread through the autosomal material, converting, at least partially, a structural autosomal trisomy into a functional autosomal disomy. Of course, there would be a partial X monosomy as well. This scenario is discussed in more detail in the section on "Double-Segment Exchange, Adjacent-2."

> A truly single-segment X-autosome translocation, the translocated segment comprising X material, is recorded in de Vries et al. (1999). A mother had a submicroscopic segment of the PAR1 in distal Xp (p22.31-pter) translocated across to the short arm of a chromosome 14, but, as far as could be seen, there was no reciprocal movement back to the X of any 14p material. She transmitted the der(X) to a son, who presented signs interpreted as consistent with nullisomy for certain genes in the distal PAR1: the *SHOX*, *MRX*, *CDPX*, and *STS* genes, their absences responsible respectively and collectively for short stature, developmental delay, short limbs, and ichthyosis.

Single-Segment Exchange, Autosomal Translocated Segment

The single segment being of autosomal origin, with only the telomeric tip of Xp or Xq translocated in exchange, is shown in the middle column of Figure **6–7**, segregations *d–g*. The imbalanced conceptions from 2:2 adjacent-1 malsegregation would be partially monosomic or partially trisomic for the autosomal segment: 46,der(autosome) and 46,der(X), respectively (segregations *d* and *e* in Fig. **6–7**). The partial autosomal trisomic state may, in the 46,X,der(X) female, have an attenuated phenotype due to spreading of inactivation from the XIC of the der(X) into the autosomal segment. The 46,Y,der(X) male conceptus, in which no X-inactivation occurs, would show the undiluted effect of the partial autosomal trisomy. The partially monosomic state, 46,XX,der(autosome) or 46,XY,der(autosome), would be no different than if the other chromosome participating in the translocation had been an autosome, instead of an X, and the typical clinical consequence associated with that autosomal deletion would be expected.

Double-Segment Exchange, Adjacent-1

In a double-segment exchange with adjacent-1 segregation (right column, Fig. **6–7**, segregations *h–i*), there may be, in the unbalanced conceptus, effects of a combined X functional disomy and autosomal monosomy, or of X monosomy (or nullisomy) and autosomal trisomy. Such combinations would often be lethal in utero. But in the 46,X,der(X) female (segregation *i*), the effects may be very considerably modified by spreading of inactivation. Consider the t(X;16) illustrated in Figure **6–9**. The 46,X,der(X) daughter has both a monosomy for most of Xp, giving a Turner-like phenotype, and a structural trisomy for most of 16p. Following spread of inactivation in the der(X) into its autosomal

segment in a fraction of cells, the 16p trisomy has been converted, in these cells, into a functional 16p disomy. In 76% of cells, however (and in the cell illustrated), the inactivation has not extended into the 16p segment. Thus, she has, effectively, a functional mosaic 16p trisomy/16p disomy. This same combination with a Y replacing the X as the intact sex chromosome, 46,Y,der(X), with nullisomy Xp/trisomy 16p, would not be viable. The other adjacent-1 conceptions with 46,XX,der(16) and 46,XY,der(16) (light arrows in Fig. **6–9**; *h* in Fig. **6–7**) would not be similarly "modifiable" and would have a very large functional imbalance, and they would also be expected to abort early in the pregnancy.

Double-Segment Exchange, Adjacent-2

Adjacent-2 segregation typically produces trisomy for much of one chromosome along with monosomy for much of the other, and this is not, in the usual autosome-autosome translocation, remotely viable (e.g., segregation (5) in Fig. 5–4). But such an enormous degree of structural imbalance can be accommodated in some X-autosome translocations, in a female conceptus. First, consider the case of the intact autosome and the derivative autosome being transmitted together: 46,X,–X,+der(autosome). Provided the X segment of the der(autosome) includes the XIC (segregation *b* in Fig. **6–8**), inactivation can spread from the XIC in both directions and into the autosomal segment, counteracting the effect of the autosomal duplication, at least partially. The concomitant partial X monosomy is, of itself, a viable state. The child would be expected to display a partial Turner phenotype, upon which the effect of a variably inactivated partial autosomal trisomy would be added. This is illustrated in Leisti et al. (1975), who record a mother carrying a t(X;9)(q11;q32) and her daughter being 46,X,–X,+der(9). In the daughter, inactivation spread through much of the autosomal segment, which very substantially, although not completely, neutralized the effect of the partial trisomy 9: she had a Turner phenotype with superadded microcephaly and mental defect. The case in Williams and Dear (1987) is similar, with a retarded and dysmorphic child having the karyotype 46,X,–X,+der(10),t(X;10)(q11;q25)mat, but in this instance inactivation into the autosomal segment was apparently blocked at the centromere of the der(10). This left the child with an effective duplication of 10p, along with the X deletion (Fig. **6–10**). Concerning a male conception in this setting, of course an adjacent-2 conceptus could not survive.

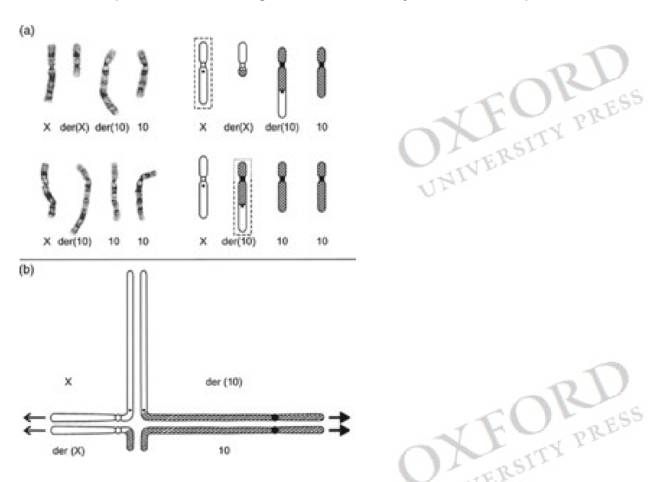




Figure 6–10
Adjacent-2 segregation. (*a*) Mother with balanced X;10 translocation (*above*), and her daughter with a 46,X,–X,+der(10) karyotype (*below*), on G-banding. The translocation is t(X;10)(q11;q25). Replication-banding showed the normal X to be inactivated in all 30 lymphocytes analyzed in the mother (dashed box on cartoon karyotype; dot indicates X-inactivation center). The daughter's der(10) was preferentially inactivated (dashed outline of box) in 50/50 cells, but the inactivation did not continue through to the 10p segment (dotted outline of box). The phenotype is the combined result of the 10p duplication and Xp monosomy. (Case of J. Williams; Williams and Dear, 1987.) (*b*) The presumed pachytene configuration during gametogenesis in the mother (X chromatin, open; chromosome 10 chromatin, crosshatched; dot indicates X-inactivation center). Arrows indicate movements of chromosomes to daughter cells in adjacent-2 segregation; heavy arrows show the combination observed in this family. This is segregation *b* in Figure **6–8**.

Second, viability is also possible in one very rare circumstance of an intact X and the der(X) being transmitted together, with the adjacent-2 karyotype 46,XX,–(autosome),+der(X), segregation *c* in Figure **6–8**. The der(X) must contain an XIC; its autosomal segment must comprise a very substantial amount of the chromatin of that autosome; and there must be little or no spread of inactivation beyond the X segment of the translocation chromosome into the autosomal segment. In this way, the autosomal component can maintain sufficient disomic genetic activity to produce a viable phenotype. Only autosomes with "genetically small" short arms could enable these criteria to be met. An example from a maternal t(X;22)(p21.3;q11.21) is shown in Figure **6–11** (daughter with adjacent-2, bottom row). The der(X) comprises most of an X and all, or almost all, of 22q. If its 22q segment were blocked from inactivation, there would be, in effect, a near-normal functional disomy 22, along with a partial XXX syndrome. In fact, this woman had a mild intellectual disability and attended a special school; the relative contributions to her phenotype of the two components of the cytogenetic abnormality are open to speculation.

Double-segment exchange, 3:1 segregation with tertiary monosomy

The same criteria noted in the earlier paragraph may also obtain in the rare situation of tertiary monosomy being viable (in essence, this is segregation *k* in Figure **6–7**). The t(X;22) in Figure **6–11** again provides an example. In the index case in this family having the tertiary monosomy state 45,X,–X,+der(X),–22 (middle row, Fig. **6–11**), the der(X) chromosome is preferentially inactivated, but inactivation has not (at least on blood lymphocytes) spread through to the 22q component of the der(X). Thus, a functional 22 disomy is maintained, or nearly so. The important structural imbalance, one might have predicted, could have been limited to the Xp21.3-pter deletion (loss of 22p being without effect), with the phenotype confined to a Turner-like picture. In the event, however, there were minor congenital anomalies, and she was assessed as being intellectually disabled. This does suggest that the pattern of inactivation elsewhere in the soma may have differed from that observed on peripheral blood, and there might have been a degree of functional 22q monosomy in other tissues, including brain.

3:1 Segregation, interchange trisomy/monosomy

From each of the categories of single- and double-segment exchange, 3:1 interchange trisomy could theoretically produce Klinefelter syndrome or XXX syndrome along with the balanced translocation; and interchange monosomy could produce standard 45,X Turner syndrome. We are aware of only one such outcome, an infertile woman with 47,XX,t(X;12) from a 46,X,t(X;12)(q22;p12) mother, the imbalance being equivalent to 47,XXX (Madan et al., 1981).

4:0 Segregation

With a trisomically viable autosome, say chromosome 21, a 48,XX,+der(X),+der(21) karyotype might be equivalent to the potentially viable 48,XXX,+21 state, a combined Down syndrome plus XXX syndrome. We know of no such report.

Male Meiosis

Meiosis in the X-autosome hemizygote is typically compromised, due to failure of formation of the sex vesicle, and spermatogenesis arrests. Infrequently, some sperm may be made, albeit in small numbers. Perrin et al. (2008) propose formation of a quadrivalent, in which the Y chromosome participates with apposition of its PAR1 and PAR2 to the homologous regions on the der(X) and the der(A) (Fig. **6–12**). As with the female, some malsegregant forms would have potential viability, due to the potential lesser effects of X imbalance. Similarly according to the principles as set out earlier for the female, but with the additional factor of a Y chromosome to be considered, the reader can determine the range of possibilities in a particular case, for this rarely encountered circumstance.

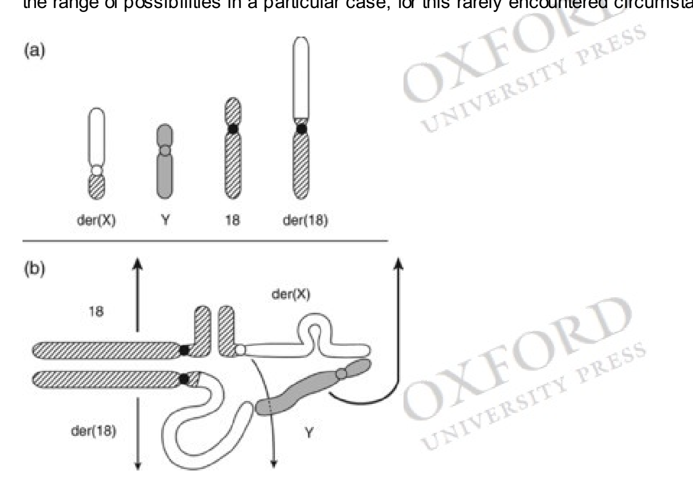




Figure 6–12

The X-autosome translocation in the carrier male, if meiosis is able to proceed. (*a*) Father with balanced X;18 translocation, from whom pregnancy was achieved following in vitro fertilization with intracytoplasmic sperm injection. (*b*) The presumed pachytene configuration during gametogenesis in the father (X chromatin, open; Y chromatin, gray; chromosome 18 chromatin, crosshatched). According to this construction, the X segments and the Y align only at the respective pseudoautosomal regions (PARs), and otherwise lie free. Heavy arrows indicate movement of chromosomes to daughter cells in one of the 2:2 alternate segregations, to produce a normal gamete, as observed in the 46,XY son. Light arrows show the other alternate combination, which could lead to a carrier daughter. (Drawn after the case in Perrin et al. 2008.)

Y-Autosome Translocations

Y-autosome translocations fall into two major Yq-breakpoint categories, one of which has important clinical implications, and the other of which does not. Certain other rare forms exist. First, some brief comments on the nature of the Y chromosome are in order.

The Role and Behavior of the Y Chromosome

The particular raison d'être of the Y chromosome is to bring about male development. The testis-determining gene, *SRY*, lies in the euchromatic region on the short arm, just 5 kb proximal to the pseudoautosomal boundary. As noted earlier, and see Figure **6–1**, the primary pseudoautosomal regions (PAR1) of the Y and X short arm contain homologous loci, and certain other loci elsewhere in the Y have homologs on the X (Disteche, 1995). The secondary pseudoautosomal region (PAR2) is located at distal Yq and distal Xq; its loss from Yq seems to be without phenotypic consequence (Kühl et al., 2001). From the point of view of reproductive health, three "azoöspermia factor regions" on Yq are of importance, named AZFa, b, and c, and these are discussed in more detail in Chapter 23. Otherwise, about half the Y—the amount is variable—comprises the genetically inert heterochromatic region of the long arm (Yq12), which contains highly repetitive DNA sequences.

Meiosis

During normal meiosis in the male, the X and Y chromosomes recombine, synapsing at the PAR1 at the tips of Xp and Yp. The two sex chromosomes joined together in this way, but otherwise unsynapsed, comprise the "sex vesicle" (or sex-body, or XY-body), which can be considered as a peripheral nuclear subdomain within which the X and Y chromosomes lie, genetically inactivated, during the pachytene stage of meiosis (Turner, 2007). A properly formed sex vesicle is necessary for normal spermatogenesis, and anything that interferes with its normal formation—such as the presence of a translocation chromosome—will compromise the process of sperm development. We have seen earlier that an X-autosome translocation in the male practically always causes spermatogenic arrest. And fairly infrequently, some autosomal translocations, and more especially those involving an acrocentric chromosome, can cause interference with the sex vesicle, with consequential infertility. Similar considerations apply to the balanced Y-autosome translocation (other than the Y-acrocentric translocation) (Maraschio et al., 1994). The autosomal components of the quadrivalent, "dragged into" the sex vesicle, as it were, can have a damaging effect, possibly due to their being inactivated, and preventing any further progress of meiosis (Turner, 2007). Delobel et al. (1998) illustrate this circumstance in their study of a phenotypically normal infertile man with a translocation t(Y;6)(q12; p11.1). They analyzed meiotic preparations from a testicular biopsy, noting the configuration of the quadrivalent, this comprising the X,der(Y),der(6), and normal chromosome 6. The autosomal elements of the quadrivalent were seen to have been drawn into the sex vesicle and to be hypercondensed. The result was spermatogenic arrest at the pachytene stage, with subsequent degeneration of spermatocytes. In a similar case, Sun et al. (2005) analyzed testicular tissue from an azoöspermic man with a Yq;1q translocation and showed (using MLH1 staining) a reduced level of meiotic recombination, with only a small fraction of cells progressing through to pachytene, and again the autosomal translocated segment drawn into the sex vesicle. Yet (and in contradistinction to the X-autosome story in the male) the Y-autosome carrier may occasionally retain natural fertility. Otherwise, fertility may be "rescueable" by means of assisted reproduction, as discussed further later.

Balanced Reciprocal Yq and Autosome[4] Translocation

Reciprocal exchange between the Y long arm and an autosome produces a balanced Y-autosome translocation. In the form being considered here, the Y breakpoint is usually given as q11.2 or q12, and the autosomal breakpoint is anywhere on the autosomal karyotype, other than an acrocentric short arm (Braun-Falco et al., 2007). There are associated phenotypic abnormalities in a few individuals, and this may be due to a disruptive effect at the breakpoints, or a deletion of autosomal material distal to the breakpoint (Erickson et al., 1995). In most, the rearrangement may be truly balanced, with the physical and intellectual phenotype being normal, and infertility is the usual presenting factor. Given this latter fact, it follows logically enough that the translocation would typically arise as a de novo event, and this is indeed the observation (Pinho et al., 2005). The infertility may be a result of disruption of the sex vesicle, as discussed earlier. In other cases, documented loss of the AZF region may explain the infertility (Brisset et al., 2005).

If spermatogenesis is able to proceed, there is a risk for the generation of unbalanced forms, and a few examples are on record. Mademont-Soler et al. (2009) describe a fertile couple, the father having the karyotype 46,X,t(Y;12)(q12;q24.33). The der(12) was deleted for a very small distal segment of chromosome 12 (12q24.33-qter), whereas the der(Y) carried this material. In two pregnancies, the two different adjacent-1 segregations were observed: 46,der(12) in one, and 46,der(Y) in the other. Both pregnancies, with thus an autosomal deletion and a duplication respectively, were terminated.

A few familial cases have been reported, with father and son having the same balanced rearrangement. Teyssier et al. (1993) document a man with severe oligoasthenospermia who had a t(Y;1)(q11;q11), and whose father proved to carry the same translocation. Intact fertility is well illustrated in the family described by Sklower Brooks et al. (1998), depicted in Figure **6–13**. One son in a sibship of five males and two females, he himself a university graduate, had presented for genetic counseling when his wife had a third miscarriage (they also had a normal daughter). The deceased father must have carried a t(Y;8)(q12;p21.3), with three sons showing the balanced state and two sons having inherited an unbalanced complement, 46,X,der(Y). The unbalanced state conferred a partial trisomy for 8p22-pter, which was associated with a mild learning difficulty.

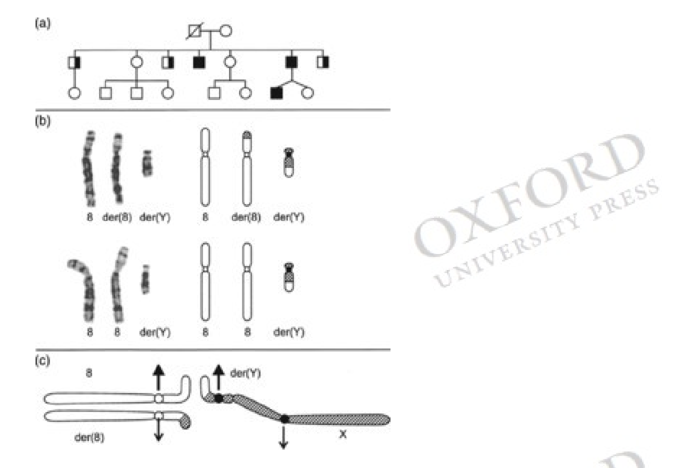




Figure 6–13
A Y-autosome translocation, not involving an acrocentric short arm. In this particular example, and somewhat unusually, fertility is apparently normal. The autosomal translocated segment is of small size, structurally and functionally, and the aneuploid state with a dup(8p) is not only viable, but associated with only a mildly abnormal intellectual phenotype and an essentially normal physical appearance. (*a*) Family tree. Filled symbol, unbalanced karyotype; half-filled symbol, balanced carrier. The deceased grandfather is presumed to have been a translocation heterozygote. (*b*) Partial karyotype of a translocation heterozygote (*above*), showing the Y;8 translocation, and one of the individuals with the unbalanced complement (*below*). (*c*) The presumed pachytene configuration during gametogenesis in the heterozygote (chromosome 8 chromatin, open; Y and X chromatin, crosshatched). Arrows indicate movements of chromosomes to daughter cells in "adjacent-1" segregation; heavy arrows show the combination observed in this family. (From S. Sklower Brooks et al., 1998, Normal adaptive function with learning disability in duplication 8p including band p22, *American Journal of Medical Genetics* 78:114–117, and with the permission of Wiley-Liss.)

With access to ICSI (intractyoplasmic sperm injection), biological paternity becomes possible for some carriers. The man with the 46,X,t(Y;18)(q11.2q21) translocation shown in Figure **6–14** had been karyotyped in the course of investigation for infertility with severe oligospermia, he being an otherwise normal person. In this Y;18 case, of the 16 possible embryos, more than half, including one of the 4:0 segregants, would in theory be viable; the reader may wish to work out which ones these might be. Only one sperm, the 23,X, is capable of producing a phenotypically and karyotypically normal child; the other gamete from alternate segregation, 23,t(Y;18), would produce a son who would likely have similar infertility. At preimplantation genetic diagnosis (PGD), the chromosomally unbalanced embryos could be discarded. With a small number of eggs retrieved on each stimulation cycle, a normal combination might well not happen, given that there are 14 unbalanced possibilities, *if* each outcome were equally likely. But in fact the observations of Sklower Brooks et al. (see earlier discussion) and Giltay et al. (see later discussion) provide some encouragement that the odds for the Y-autosome carrier (in other words, the meiotic predisposition) may be tipped in favor of the normal and balanced forms. As it turned out in this Y;18 case, one embryo was indeed 46,XX, and this was successfully implanted.

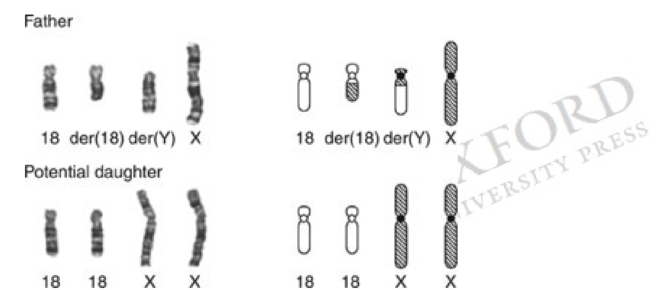




Figure 6–14
The Y-autosome translocation and infertility. This t(Y;18)(q11;2q21) was identified in a man presenting with oligospermia during investigation for infertility. The fact that some sperm are still being produced allows the option of in vitro fertilization with intracytoplasmic sperm injection. A considerable number of these unbalanced gametes could, in theory, be viable. Only a 46,XX daughter could be both karyotypically and phenotypically normal. (Case of L. Harris and L. Wilton.)

This question is more directly answered in Giltay et al. (1999), these workers undertaking a sperm analysis in a man with a t(Y;16)(q11.21;q24). Sperm were present, but few in number, with many abnormal forms (oligoasthenoteratozoöspermia). Although alternate segregation accounts for only 2 out of the 16 segregation possibilities, in fact in this case half of all morphologically normal spermatozoa were normal or balanced, with about 40% showing adjacent segregation, and about 10% with 3:1. But the fractions were less favorable if abnormal sperm were included. With reference to assisted conception and using ICSI, Giltay et al. speak of in vitro and in vivo selection (the former artificial, the latter natural) combining to effect a considerable reduction in the risk for an unbalanced offspring. In fact, this man had had three children by ICSI, two normal daughters and a carrier son.

Y Long-Arm and Acrocentric Short-Arm Translocations

Yqh Material on Acrocentric Short Arm, 46-Chromosome Count

This is the most common form of Y-autosome translocation, accounting for some 70% of cases. The autosome is one of the acrocentrics, most commonly chromosome 15, followed by chromosome 22. There is no loss or gain of euchromatin; the result is that one acrocentric carries some phenotypically irrelevant Y heterochromatin, looking rather like (and sometimes mistaken for) a very long short arm (Neumann et al., 1992). The breakpoints are sited in the acrocentric short arm (p11-p13) and in the heterochromatin of

the Y long arm (Yq12) (Fig. **6–15**). Males and females can equally be carriers. Neither phenotypic abnormality nor infertility appears to be associated (Hsu, 1994). Rare disquieting reports need to be viewed cautiously, such as that of Rajcan-Separovic et al. (2001), who raise the possibility of a secondary chromosomal abnormality, in documenting the remarkable instance of a woman with the karyotype 46,XX,der(15)t(Y;15)(q12; p13)mat, of older childbearing age, who had had two trisomic 15 pregnancies.

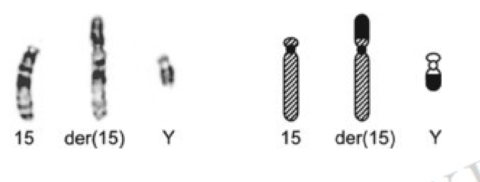


Figure 6–15
An example of the Y-autosome translocation involving an acrocentric autosome, the der(15) depicted here, with the breakpoint in the acrocentric short arm. Normal chromosome 15 and normal Y shown alongside for comparison (chromosome 15 chromatin, crosshatched; Y heterochromatin, filled; Y euchromatin, open). The translocation chromosome can be carried equally by males and females. The karyotype appears unbalanced, but the phenotype is normal.

Rare Forms

Yqh Material on a Nonacrocentric Chromosome, 46-Chromosome Count

If Yq heterochromatin is translocated to the tip of an autosome, other than to an acrocentric short arm, there may or may not be reproductive implications. A der(1)t(Y;1)(q12;p36) in a French family could be traced back to a common couple married in 1773, with self-evident fertility, male and female, for more than two centuries (Morel et al., 2002a), and Vozdova et al. (2011) showed normal seminal indices in a man with a familial der(4)t(Y;4)(q11.23;p16.3).

Y Material Plus Acrocentric Short Arm Tip, 46-Chromosome Count

This is essentially the countertype of the common case exemplified by the Y;15 described earlier, in which the other reciprocal product, the der(Y), replaces a normal Y. Hoshi et al. (1998) identified a perfectly normal man, the father of three, who had a 46,X,–Y,t(Y;15)(q12;p13) karyotype. The der(Y) contained the necessary male-determining and fertility regions. He was only investigated because his sister had a gonadal tumor of testicular origin, she having the mosaic karyotype 46,X,Y,t(Y;15)/45,X.

Y Material to Autosome Translocation, 45-Chromosome Count, Including "45,X Male".

The testis-determining region of the Y, containing the *SRY* gene, can be translocated onto an autosome, usually an acrocentric (Farah et al., 1994). The individual, phenotypically male, has 45 chromosomes, including the Y+autosome fusion product. The translocated Y segment may be beyond the level of cytogenetic resolution, and the karyotype can appear as 45,X ("45,X male") until further studies cast light (C-P Chen et al., 2008). The translocation may be of no phenotypic or reproductive effect, as Callen et al. (1987) record in a family identified quite by chance, in the course of a research study, in which a man and two sons had the karyotype 45,X,dic(Y;22)(q11.23;p11.2). A similar story is presented in Morales et al. (2007), in this case the karyotype being 45,X,psu dic(Y;22)(qter;p11.2), with the Y+22 chromosome comprising almost an entire Y and almost an entire 22. The chromosome very evidently did not affect fertility in 10 carrier fathers in this large family; this may have reflected that recombination of the PAR1 of the Y with that of the X was not obstructed, due to the chromosome 22 component being well "out of the way" of the sex vesicle, so to speak. The active centromere of the Y+22 chromosome was from the chromosome 22, the Y centromere being inactivated, and thus it was segregation of the 22 chromosomes that was the sex-determining mechanism in the offspring of these men. It was interesting that only normal children were born, whereas, in theory, segregation might also have led to 45,X Turner syndrome and 46,XX,psu dic(Y;22) Klinefelter syndrome.

More often, the reproductive and sometimes the physical phenotype is affected. Azoöspermia is a frequent finding, as documented in a review of 31 cases of "45,X male" (C-P Chen et al., 2008). The Y component may be translocated insertionally, as Yenamandra et al. (1997) demonstrated in a phenotypically abnormal "45,X" boy, in one of whose chromosomes 4, at 4p15.3, the *SRY*-bearing segment was accommodated. Rather more obvious cytogenetically was the de novo dicentric Y;13 translocation, 45,X,dic(Y;13)(p11.3;p11.2), described in Shanske et al. (1999a): the translocation comprised almost a complete 13+Y composite. Their patient was a very short and otherwise normal 10-year-old boy, in whom the *SHOX* growth control gene, normally located in the PAR1, was absent. A patient with a very similar karyotype reported in Alves et al. (2002) was a man, 170 cm tall, with severe oligospermia.

Yp;15q Translocation and Prader-Willi Syndrome.

A very few cases of Prader-Willi syndrome (PWS) have been due to a fusion between a Y and a chromosome 15, having the karyotype 45,X,t(Y;15) with varying breakpoints, either de novo or familial (Vickers et al., 1994; Puvabanditsin et al., 2007).

A remarkable example concerns a familial t(Y;15)(p11.2;q12), described in Gole et al. (2004). The father with the balanced translocation had a daughter with presumed PWS, she having inherited his X chromosome and the der(Y) which comprised almost all of 15q but lacking the PWS region, and most of the Y but lacking *SRY*. Her karyotype 46,XX,–15,+der(Y) reflected what might be called a "version" of adjacent-2 segregation. The absence of a paternally originating PWS region led to the development of that syndrome, while the absence of *SRY* was the basis of female sex. Her brother had the countertype "standard" adjacent-2 combination, 46,X,–Y,+der(15): he was of below-average intelligence, presumably due to duplication of the proximal 15q region, 15cen→q12, and had required treatment for hypogonadism

The De Novo Yq;1q Translocation With 1q Trisomy.

Five cases are on record of a de novo unbalanced translocation t(Y;1)(q12;q21), mostly seen in mosaic state; this translocation comprises a majority of the total of eight known cases with an essentially complete 1q trisomy (Scheuerle et al., 2005). Presumably, similar sequences on 1q21 and Yq12 predispose to this recurring rearrangement. The phenotype, unsurprisingly, is very severe.

X-Y Translocations

The Classical X-Y Translocation

Of the major types of X-Y translocation, the classical form is the most frequently seen (Fig. **6–16a, b**). The X and Y breakpoints are constant, at the cytogenetic level, involving Xp22.3 in the distal X short arm, and Yq11/q12 in the proximal Y long arm (Bukvic et al., 2010). It is, certainly, readily recognized cytogenetically and has the karyotypic notation 46,X or Y,der(X),t(X;Y)(p22.3;q11). The important genotypic defect is deletion of the distal Xp segment, with the loss including PAR1. At the molecular level, there is variation in the amount of Xp deleted, and the phenotype depends in part at least upon which of the following distal Xp genes may be lost: *ARSE* (arylsulfatase E, for chondrodysplasia punctata), *SHOX* (short stature homeobox, for Leri-Weill dyschondrosteosis), *STS* (steroid sulfatase, for ichthyosis), *KAL* (Kallmann syndrome), *MRX49* and *NLGN4* (mental retardation), and *OA1* (ocular albinism). The Y-originating segment does not contain *SRY*. The (female) person who is 46,X,der(X)t(X;Y) has a partial monosomy for this Xp segment, and the (male) individual with 46,Y,der(X)t(X;Y) is partially nullisomic.

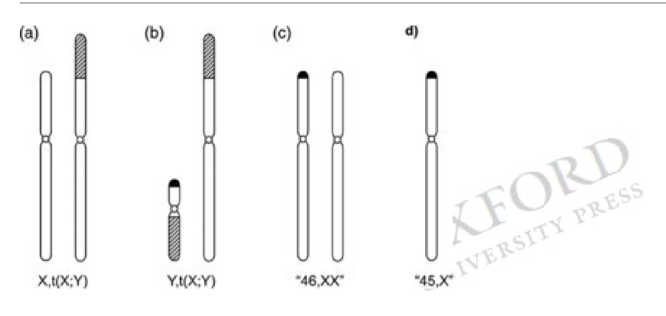




Figure 6–16
Four more frequent ways in which X-Y translocations are seen. (*a*) The classical t(X;Y)(p22.3;q11) together with a normal X (in a female). (*b*) The classical t(X;Y) together with a normal Y (in a male). (*c*) The cryptic t(Xp;Yp), with the Yp segment containing the *SRY* gene, in a "46,XX male." (*d*) The cryptic t(Xp;Yp) as the sole gonosome, in a "45,X male." Y chromatin: white indicates Y euchromatin, black indicates that part of distal Yp euchromatin encompassing the pseudoautosomal region and the SRY locus, and crosshatching indicates Yq heterochromatin. Note that gonadal sex accords with the absence (*a*) or presence (*b–d*) of the SRY gene.

The *female* t(X;Y) heterozygote is characteristically fertile and of normal intelligence. If *SHOX* is deleted, the monosomic state for this gene determines a particular form of short stature and wrist deformity (Leri-Weill dyschondrosteosis) (Calabrese et al., 1999; Bukvic et al., 2010). The *male* t(X;Y) hemizygote is typically the son of a t(X;Y) mother (Hsu, 1994). Some may be cognitively normal, in those in whom the breakpoint is more distal. Should a more proximal molecular breakpoint expose a mendelian "brain gene," such as *MRX49* and *NLGN4* mentioned earlier, mental impairment results, and apparently this is more usually the case. If the male has Leri-Weill dyschondrosteosis, it is no more marked than in the female, reflecting the fact that the *SHOX* locus is in the PAR1, and each sex still retains one copy of the gene, on their normal X or Y, respectively. Infertility is almost invariable, due to spermatogenic arrest (Gabriel-Robez et al., 1990).

Sperm production has, however, been documented in one case, albeit at a very low level (125,000 per ml), in a man with the typical 46,Y,der(X),t(X;Y)(p22.3;q11) karyotype (Morel et al., 2001). He was of normal intelligence, height 165 cm (5 feet 5 inches), with normal external genitalia and normal endocrine indices, and he had presented with infertility. There were equal numbers of 23,der(X) and normal 23,Y sperm, but about 20% of sperm were otherwise abnormal, the most common defect being 24,Y,der(X).

The majority of cases are familial. Presumably, the X-Y chromosome arose following a reciprocal exchange between the X and Y during spermatogenesis in the individual fathering the originating (female) translocation carrier in the family. This event is facilitated by the apposition of X and Y segments having a high degree of homology; for example, a crossover between the Kallmann locus on the X chromosome and a Kallmann-like nonfunctional pseudogene on the Y chromosome long arm (Guioli et al., 1992). In the female, the pattern of X-inactivation tends toward inactivation of the der(X)t(X;Y), but this is variable and unpredictable (Gabriel-Robez et al., 1990).

Some X-Y translocations cytogenetically apparently identical to the classical type are associated with the rare syndrome of microöphthalmia with linear skin defects (MLS) (Kotzot et al., 2002). The phenotype results from aberrant expression of the *HCCS* gene at Xp22, which encodes a synthase producing cytochrome c, better known as one of the components of the mitochondrial respiratory chain, but which also has a role in inducing apoptosis (Wimplinger et al., 2006). Disordered apoptosis may be the basis of the tissue defects in MLS.

The Cryptic Xp-Yp Translocation ("XX Male" and "45,X Male")

This form of the X-Y translocation is usually not visible (or barely visible) to the cytogeneticist without the use of fluorescence in situ hybridization (FISH) using Yp sequences as probe. Again, the X breakpoint is within Xp22.3; but the Y breakpoint is in the short arm, proximal to the testis-determining gene (*SRY*). The genotypic consequences are loss of the distal region of the X chromosome and the transfer of the *SRY* gene onto an almost intact X chromosome. Thus, the person is male. The karyotype would initially appear to the cytogeneticist either as 46,XX or as 45,X (Fig. **6–16c, d**). In truth, it is 46,X,der(X)t(X;Y)(p22.3;p11) or 45,der(X)t(X;Y)(p22.3;p11). This translocation accounts for most supposed XX males and some 45,X males. If there is loss of one copy of the *SHOX* gene, Leri-Weill dyschondrosteosis (see earlier) is the expected consequence (Stuppia et al., 1999). The MLS syndrome, mentioned earlier, has been observed (Anguiano et al., 2003). The translocation arises from an abnormal X-Y recombination during paternal meiosis (Weil et al., 1994). Almost always, it occurs sporadically, and the affected males are infertile, although an extraordinary familial exception is recorded in Sharp et al. (2004). Two individuals, likely distantly related, presented with ovotesticular disorder of sex development. It may be that *SRY* in these two was partially operating, due to a variably penetrant position effect.

"46,X,d el(Yq)" With Cryptic Xq-Yq Interchange

A third category is the X-Y translocation arising de novo from an exchange during paternal spermatogenesis between Yq and distal Xq, producing an apparent del(Yq) chromosome that actually contains a very small segment of distal Xq (Lahn et al., 1994). The karyotype could be written 46,X,der(Y)t(X;Y)(q28;q11.2). The functional distal Xq disomy produces a characteristic severe phenotype (Sanlaville et al., 2009). In the female, the reciprocal 46,X,der(X) karyotype might hypothetically imply a mild phenotype, essentially reflecting a very small distal del(Xq), and assuming that the abnormal X would be preferentially inactivated. Cheng et al. (2009) provide a rare example of an Xq;Yq female, a 33-year-old woman who presented with premature ovarian failure, and whose karyotype they determined to be 46,X,der(X)t(X;Y)(q26.3;q11.223). The X chromosome could be appreciated as subtly abnormal on classical cytogenetics; it took comparative genomic hybridization (CGH) to discover the Yq material, and then FISH to reveal the true nature of the rearrangement.

Other Variant Forms

Other forms of t(X;Y), typically but not always arising de novo, are often associated with infertility, and in some with intellectual deficit. The possibilities are listed and illustrated in Hsu (1994) and include der(X) chromosomes from translocations of varying lengths of Yq to a breakpoint at various levels on Xp or Xq, and der(Y) chromosomes from translocations of varying small lengths of Xp to a breakpoint at various levels on Yq. The dicentric X-Y translocation comprises an almost complete Y attached at a distal Yp breakpoint to an X chromosome, at either an Xp or an Xq breakpoint. For example, Baralle et al. (2000) describe a girl with a Yp to Xp rearrangement, the karyotype 46,X,dic(X;Y)(p22.3;p11.2), who presented with Leri-Weill dyschondrosteosis. Her pubertal development was regarded as being normal, although she had yet (at age 14 years) to undergo menarche. Her femaleness was due to absence of the SRY gene, the breakpoint on Yp being proximal to its locus. A girl who presented at age 10 with an ovarian cancer, and in whom the X breakpoint was in the proximal long arm, her karyotype 46,X,der(Y)t(X;Y)(q13.1;q11.223), is reported in Lissoni et al. (2009); her female gender may have reflected inactivation of *SRY* from the influence of the XIC upon the der(Y).

X-X Translocations

The general karyotype is written 46,X,der(X), t(Xp;Xq), and the resultant imbalance is a dup/del of Xp/Xq, or vice versa. The translocation could have arisen following unequal recombination between the two X chromosomes in the oöcyte. Or the rearrangement could have occurred within the one X chromosome, folding in upon itself, in which case the origin is more likely paternal (Giglio et al., 2000). Xp11.23 and Xq21.3 are favored as breakpoints, and the translocated del/dup segments may therefore be large.

Pubertal and/or menstrual abnormality is the usual presentation, and infertility is the rule (Letterie, 1995). Maternal transmission is, however, recorded in Reinehr et al. (2001),

concerning a mother and daughter with short stature both having a t(X;X)(p22;q26) chromosome. These breakpoints are distal, and thus the del/dup segments in this case are small. The monosomic Xp segment included the *SHOX* gene, and this presumably was the cause of the short stature. The mother had had a normal menstrual history, and she had two other healthy children normal heights.

Y-Y Translocations

For the sake of completeness, the presumed existence of the very rare Y-Y translocation is noted. Some may in fact have been isodicentric Yq chromosomes (Hsu, 1994; Hernando et al., 2002).

Genetic Counseling

The X-Autosome Translocation

Fertility is affected in the X-autosome heterozygote and hemizygote. Approximately half of the female carriers, and practically all males, are likely to be infertile.

The Female Heterozygote

If fertile, the female heterozygote has a substantial risk for having abnormal offspring due to an imbalanced chromosomal constitution. At one end of the scale, the abnormality might be mild (e.g., partial Klinefelter syndrome) or barely discernible (e.g., partial X trisomy). At the other end, it could be severe (e.g., partial X disomy or autosomal aneuploidy, not modified by inactivation). The counselor should determine the theoretical gametic combinations from the particular category of translocation, with reference to the examples described in the section on "Biology." Adjacent-1 and 3:1 tertiary trisomy are the major malsegregation modes to be considered. Figures **6–7** and **6–8** provide a guide; but each translocation needs to be assessed on its own merits. General comments follow.

(1) A single-segment translocation with an X segment of *large* size would imply risks for partial Turner, partial Klinefelter, and partial XXX syndromes (Figs. **6–2** and **6–4**; Fig. **6–7b, c**).
(2) A single-segment translocation with an X segment of *small* size would imply a risk not only for one of these three partial gonosomal aneuploidies but also for functional disomy or nullisomy for a small distal Xp or Xq segment. A functional disomy would have a severe outcome (Fig. **6–5b**, segregation as per heavy arrows; Fig. **6–7a**). Nullisomy in the male, for all but the smallest segments, would be lethal in utero (Fig. **6–5b**, segregation as per light arrows; Fig. **6–7b**). The borderline between viable (but severe) and nonviable Xp deletion in the male may be at Xp22.2, in which about 10 Mb of DNA is removed (Melichar et al., 2007).
(3) A single-segment translocation with an autosomal translocated segment of "viable size" (Fig. **6–7d–f**) implies a risk for partial autosomal monosomy or trisomy from adjacent-1 segregations. In the female conceptus, the trisomy may be modified by spreading of inactivation, but this is unpredictable.
(4) Any 2:2 unbalanced segregant from a double-segment translocation (Fig. **6–7h– j**) has a combined duplication/deficiency, and spontaneous abortion is probable. But spreading of inactivation in a female conception may attenuate a partial autosomal trisomy and allow for survival, albeit with phenotypic defect.
(5) Adjacent-2 possibilities need individual assessment (Fig. **6–8**).

The Level of Risk

The risk for many female heterozygotes, who are fertile, will be "substantial." An otherwise nonviable unbalanced conception may survive because inactivation tempers the imbalance; and some conceptions with the structurally balanced complement may be functionally unbalanced due to aberrant inactivation patterns. The counselor should go through the exercise of determining possible malsegregant outcomes, as depicted in Figures **6–7** and **6–8**. The risks to have a liveborn child with a structural and/or functional aneuploidy may be in the range 20% or higher. In Stene and Stengel-Rutkowski (1988), and with specific reference to single-segmental translocations involving Xp, the risk for adjacent-1 malsegregants was 24%, although interestingly the risk associated with 3:1 segregation leading to interchange trisomy X was very low, less than 0.8%. As we discussed earlier, the components making up the total risk may comprise a very mild abnormality through to severe mental and physical defect. There is the difficulty of knowing what risk might apply to a child with the same balanced translocation (see later discussion). Only with the 46,XX and 46,XY karyotype can one be confident of normality, other things being equal.

Even more than with the common autosomal translocation, the risks relating to each X-autosome translocation will be specific to that particular rearrangement, and extrapolation from other translocations will be fraught. Panasiuk et al. (2004) have made a start, in deriving specific risk figures for four different translocations, in each case the X breakpoint involving the short arm (Table 6–2). The one circumstance in which they consider data pooling to be permissible is in rearrangements in which the autosomal breakpoint is in the short arm of an acrocentric.

Table 6–2. Estimated Risk Figures for Having a Liveborn Aneuploid Child, or a Child Stillborn or Dying as a Neonate[a], Because of Imbalance due to X-Autosome Malsegregation (Adjacent and/or 3:1), in Four Specific Translocations, Three Double Segment, and One, the X;22, Effectively Single Segment[b]

TRANSLOCATIONS	RISK[c] (%)
t(X;5)(p22.2;q32)	4.2
t(X;6)(p11.2;q21)	3.3[d]
t(X;7)(p22.2;p11.1)	2.1
t(X;22)(p22.1;p11.1)[e]	17

[a] Figures may be considered as expressing the percentage risk to have an aneuploid liveborn or stillborn infant, from a pregnancy which had proceeded to at least 28 weeks gestation.

[b] Families published or cited in Panasiuk et al. (2004).

[c] The figure in one family (X;22), in which the autosomal breakpoint is in the p arm of an acrocentric chromosome, comes from direct segregation analysis, and combining with literature cases of another X;acrocentric p arm translocation (of chromosome 15). In the remaining three, the figure is indirect and derived from applying this rule: halving the risk for the lesser of the two risks, which would otherwise have applied to each translocated segment when viewed as a single-segment imbalance.

[d] This carrier mother had presented having had an unkaryotyped malformed stillbirth at 42 weeks gestation, suggesting that at least one of the malsegregant combinations might be compatible with survival to term. The figure of 3.3% might thus be an underestimate, if the risk figure were taken to include stillbirth.

[e] This translocation is very close to, and might possibly be the same as, the t(X;22) in Figure **6–11**.

The Male Hemizygote

Infertility is almost inevitable, barring the possibility of medical intervention (Ma et al., 2003). If sperm can be accessed, from an ejaculate or via testicular sperm extraction, in vitro fertilization (IVF) using ICSI may be attempted; greater spermatogenic success may attend translocations with pericentromeric or centromeric breakpoints (Perrin et al., 2008). The outcomes from which normality could be expected, other things being equal, are the two alternate segregations leading to 46,XY, and to 46,X,t(X;A), respectively; the likelihood for this may be about 50% (as extrapolated from sperm data in one case; Perrin et al., 2008). Normality in a (necessarily heterozygous) daughter would require inactivation to have been skewed in favor of the normal X; the likelihood for this to have happened may be greater in the case of larger translocated segments. Phenotypic normality in the hemizygous father would allow the inference of a truly balanced rearrangement, and thus no question should arise about a cryptic deletion/duplication.

The presumption of an approximately 50% risk was, for example, brought to the attention of the man with an (X;18)(q11;p11.1) translocation noted earlier (see “Biology,” and Fig. **6–12**); nevertheless, and given the practical matter of a long wait for PGD, the couple went ahead with IVF and ICSI, having prenatal diagnosis in the pregnancy, and they had a normal 46,XY son (Perrin et al., 2008). Ma et al. (2003) describe a successful outcome in a man with a whole-arm translocation, t(X;20)(q10;q10), maternally inherited, and from whose ejaculate only about 50 sperm were able to be retrieved; an embryo created at IVF went on to become a carrier daughter, who was normal physically and developmentally on assessment at age 12 months. The child, and her heterozygous paternal grandmother, both displayed skewed X-inactivation. Interestingly, the man's brother, also an X;20 hemizygote, had had 7 years of infertility, but then had two normal daughters. These girls' karyotypes, and paternity status, had not been evaluated.

The balanced inherited x-autosome detected prenatally.

This can be a vexed question. Consider the circumstance of a phenotypically normal carrier mother, who has prenatal diagnosis, or PGD. A balanced X-autosome karyotype identified in a female fetus, or in a female embryo (the balanced translocation, and normal states not routinely distinguishable), might well eventually lead a normal daughter, but this cannot be made as a firm statement. As yet, we lack a good understanding of the relative roles of aberrant X-inactivation, versus gene disruption at a breakpoint, in causing phenotypic abnormality. If it were the former, then a normal carrier mother (and she having a “perfect” 100:0 normal: derivative X-activation ratio) could have an abnormal carrier daughter, if the daughter's inactivation ratio were “imperfect.” The case in Figure **6–9** might exemplify this scenario (or might not, if the child's abnormality had been coincidental). But if the latter, then the mother's normality would, of itself, indicate absence of gene disruption; and presumably her daughter would inherit the same intact translocation, and herself be normal, other things being equal. There is a need for a large study, with unbiased ascertainment, of the carrier daughter offspring of the (normal) carrier mother, in order to address this issue.

A most remarkable scenario is that of “incorrect” inactivation of the der(X) comprising the “first hit” in a tumor cascade. A mentally handicapped woman in her twenties developed schwannomas, and she was found to carry a de novo t(X;22)(p21.3;q11.21); these breakpoints are very close to the t(X;22) discussed elsewhere and shown in Figure **6–11**. Although the X-inactivation pattern on blood was appropriate, in tumor tissue the der(X) was inactivated (Bovie et al., 2003). This inactivation may have spread through to the 22q-segment, which contains two loci associated with schwannoma susceptibility (the *NF2* and *SMARCB1* loci).

Too little information exists concerning the phenotype of the male hemizygote born to a female X-autosome heterozygote for any firm advice to be offered. Normality has been recorded in this setting, but so has major genital defect, which in one case was the consequence of compromised function of the androgen receptor gene (Buckton et al., 1981; Kleczkowska et al., 1985; Callen and Sutherland, 1986; Ma et al., 2003). Fetal ultrasonography may be useful to check for normal male genital development. This approach was offered to the mother whose karyotype appears in Figure **6–11**, and who had a 46,Y,t(X;22) result at amniocentesis in her second pregnancy. A normal baby boy was subsequently born, whose infant development was quite normal. Otherwise normal male carriers would almost certainly be infertile (see earlier).

Y-Autosome Translocations

The Apparently Balanced Y-Autosome Translocation

It is notable that the same balanced Y-autosome translocation can behave differently in different male members of a family in terms of fertility, this presumably reflecting the importance of the background genetic contribution to the control of the mechanics of spermatogenesis (Teyssier et al., 1993; Rumpler, 2001). For those who are fertile, risk data are too few to form a secure base for genetic counseling. From first principles, unbalanced forms are probable, several of which will often be viable (according to the autosome in question, and the site of the autosomal breakpoint), and the option of prenatal diagnosis is appropriately offered or, and especially if IVF is needed, PGD.

As discussed in the “Biology” section, in spite of there being several more imbalanced than balanced possibilities, there are tentative grounds for supposing that alternate segregations (normal and balanced forms) may be favored. The t(Y;8) family of Sklower Brooks et al. (1998) noted earlier and shown in Figure **6–13** demonstrated three of the four predicted alternate and “adjacent-1” karyotypic outcomes: 46,XX, the 46,X,t(Y;8) balanced carrier, and 46,X,der(Y), the former two outnumbering the latter. The 46,X,der(Y) karyotype produced sons with an 8p duplication; the other unbalanced karyotype, 46,XX,der(8), would have produced a daughter with an 8p deletion. Manifestly, the carrier male, while he could have a normal daughter, could never conceive a 46,XY child. Sperm karyotyping, if available, may be a helpful investigation. In the man with the rare 13p;Yp fusion mentioned earlier (Alves et al., 2002), having demonstrated that most sperm had a balanced complement, reassurance could be offered, in this particular case, that if pregnancy were achievable there would be a good chance of producing a normal child.

For the infertile man, assisted reproduction may offer the possibility of paternity. A sperm count way below the level needed for natural conception may yet allow retrieval of sperm for ICSI. Testicular aspiration may provide sperm even when they are completely absent in the ejaculate. With the need for IVF, PGD becomes attractive because of the probable substantial genetic risk, in most cases, for unbalanced forms, and considering the practical point that the embryo is nicely accessible. Taking the example of the oligospermic man with a 46,X,t(Y;18)(q11.2;q21) karyotype, shown in Figure **6–14**, he could, in theory, and through IVF, have a 46,XX daughter, and a 46,X,t(Y;18) son like himself. The substantial fraction of unbalanced forms that could be viable in this case, out of the 16 total possible conceptions, does becomes a relevant matter at PGD. These issues of IVF and PGD are discussed in more detail in Chapter 26.

The Yqh-Acrocentric Translocation

Probably, these translocations can be regarded as being no more than interesting variant chromosomes, and of no clinical significance. In the case of the t(Yq;15p), a theoretical risk for trisomy 15 with correction to UPD (White et al., 1998; Rajcan-Separovic et al., 2001) is neither to be completely ignored nor to be overstated.

The “45,X” Yp-Acrocentric Translocation.

These chromosomes are probably stable, and not (if fertility is achievable) implying a risk for phenotypically abnormal offspring (Callen et al., 1987).

The Classical X-Y Translocation

The female with an X-Y translocation is usually fertile and of normal intelligence. She has a 50% risk for having a child, whether a son or daughter, who would have the translocation. An X-Y translocation son may be abnormal, according to the extent of distal Xp nullisomy and the loci involved (Seidel et al., 2001). If the mother is short, an X-Y translocation daughter would also be short, likely because of deletion of the *SHOX* locus. As with Turner syndrome, growth hormone treatment may be appropriate for such a child. She would probably be, like her mother, fertile. A child receiving the mother's normal X would of course be normal, 46,XX or 46,XY. Prenatal diagnosis is appropriately offered.

The male X-Y translocation carrier is almost invariably infertile. A sperm chromosome study has been undertaken in only one der(X)t(X;Y) man, referred to in the “Biology”

section earlier (Morel et al., 2001). He had severe oligozoöspermia, and notably sex chromosome aneuploidy was recorded in 20% of sperm. Otherwise, 40% of sperm were normal 23,Y, and 40% had the t(X;Y). Conception in such a case could only ever be achieved via IVF. If preimplantation diagnosis were to be attempted, the choice of a 46,XY embryo (the only normal gonosomal possibility) would allow avoiding the genetic risk for the next generation.

X-X Translocations

Infertility is the expectation, and a theoretical question of genetic risk will usually be academic. In a small imbalance, fertility may be retained, as in the example of Reinehr et al. (2001) discussed earlier. The genetic risk would, in essence, be the same as for the 46,X,abn(X) heterozygote. A daughter receiving the X-X translocation would be expected to have a phenotype similar to that of her mother. A male pregnancy would be very likely to miscarry at any early stage, due to an X nullisomy/disomy. If the del/dup segments were very small, viability might be possible, but with probable major phenotypic defect. Children receiving the mother's normal X chromosome would of course be normal, other things being equal.

Notes:

[1] Denoting the one from her mother as X^m, and the one from her father as X^p.

[5] An actual example of this sort of process, although occurring at a postnatal rather than an early embryonic stage of life, confined to one tissue (hematogenous), and at a mendelian rather than a chromosomal level, is given in Martinez-Pomar et al. (2005). These workers studied a girl with the syndrome of incontinentia pigmenti and immunodeficiency, due to the *NEMO* locus on the X chromosome. From age 2 to 4 years, her X-inactivation status in peripheral blood progressed from random to completely skewed in favor of the X with the normal *NEMO* gene, and in parallel there was correction of her immune function.

[2] Sometimes referred to as POF1 and POF2, for premature ovarian failure regions.

[3] And presumably a functional trisomy for the pseudoautosomal region within this segment.

[4] Other than an acrocentric short arm.







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Robertsonian Translocations

Chapter: Robertsonian Translocations
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THE AMERICAN INSECT CYTOGENETICIST W. R. B. Robertson first described translocations of chromosomes resulting from the fusion of two acrocentrics in his study of insect speciation in 1916, and this type of translocation is named Robertsonian (abbreviation *rob*) in his honor. There are five human acrocentric autosomes—chromosomes 13, 14, 15, 21, and 22 (the 13, 14, and 15 are the D group chromosomes, and the 21 and 22 comprise the G group)—and all are capable of participating in this type of translocation. The composite chromosome produced includes the complete long arm chromatin of the two fusing chromosomes, although it lacks at least some of the short arm chromatin. Robertsonian translocations are among the most common balanced structural rearrangements seen in the general population with a frequency in newborn surveys of about 1 in 1000 (Blouin et al., 1994). Historically, the most important Robertsonian translocations are the D;21 and G;21, which are the basis of most familial translocation Down syndrome. Uniparental disomy is of relevance, with respect to the two imprintable acrocentrics, chromosomes 14 and 15.

In this chapter, we consider the case of the phenotypically normal person who carries, in balanced form, a Robertsonian translocation. We generally use a short cytogenetic description for the carrier state, thus, 45,XX,rob(14q21q) or simply rob(14q21q). The formally correct ICSN designation for a short arm to short arm fusion Robertsonian translocation is, for example, 45,XX,der(14;21)(q10;q10) or 45,XX,rob(14;21)(q10;q10).

Biology

The great majority of balanced Robertsonian translocations involve two different chromosomes (a *heterologous* or *nonhomologous* translocation); those involving the fusion of homologs (*homologous* translocation) are very rare. Heterologous translocations can be transmitted through many generations of phenotypically normal heterozygotes, whereas the homologous translocation is almost always seen as a de novo event in the consultand. As Table 7–1 attests, the rob(13q14q) and the rob(14q21q) are predominant. If we exclude the rob(21q21q)—most of which are actually isochromosomes for 21q—the rob(13q14q) accounts for around 75% of all Robertsonian translocations in unbiased studies, and indeed it is the commonest single chromosome rearrangement in the human race. Since 1 in 1000 persons is a rob heterozygote, the prevalence of the rob(13q14q) carrier is about 1 in 1300. Karyotypes of the 13q14q and 14q21q carrier states, and of the unbalanced 14q21q state leading to translocation Down syndrome, are shown in Figures **7–1** through 7–3. Balanced carriers for any of the five homologous translocations seem to be of about equal rarity.

Table 7–1. The Frequency of Robertsonian Translocations

TRANSLOCATION	LITERATURE REVIEW	UNBIASED ASCERTAINMENT
13q13q	3%	2%
13q14q	33%	74%
13q15q	2%	2%
13q21q	2%	1%
13q22q	1%	2%
14q14q	½%	–
14q15q	2%	5%
14q21q	30%	8%
14q22q	1%	2%
15q15q	2%	–
15q21q	3%	½%
15q22q	½%	1%
21q21q*	17%	3%
21q22q	2%	½%
22q22q	1%	–

Note: Relative frequencies in literature review (most cases being of biased ascertainment), and in studies in which ascertainment was unbiased.

* Most are i(21q) Down syndrome; the figure for true rob(21q21q) is probably nearer ½%.

Source: From Hook and Cross (1987b) and Therman et al. (1989).

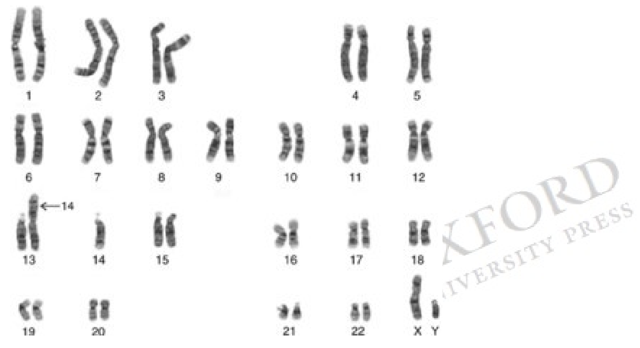


Figure 7–1
The balanced rob(13q14q) in a phenotypically normal male.



Formation of the Translocation

There are three possible mechanisms of formation of the balanced *heterologous* translocation: fusion at the centromere (centric fusion), union following breakage in one short arm and one long arm (essentially, a whole-arm reciprocal translocation), and union following breaks in both short arms (Guichaoua et al., 1986) (Fig. **7–4**). The first two mechanisms are rare (if ever, in the case of centric fusion) and would produce a translocation chromosome with one centromere (monocentric), while the third results in a chromosome with two centromeres (dicentric). The common rob(13q14q) and rob(14q21q) translocations are practically always dicentric and are formed predominantly during female meiosis, with consistent breakpoints at the molecular level (Bandyopadhyay et al., 2002). In some dicentrics, one centromere is "suppressed," and the chromosome appears monocentric. This heterogeneity of formation is not of any clinical significance that can presently be discerned. In the reciprocal type, the other product may rarely survive as a stable small bisatellited marker (Schmutz and Pinno, 1986).

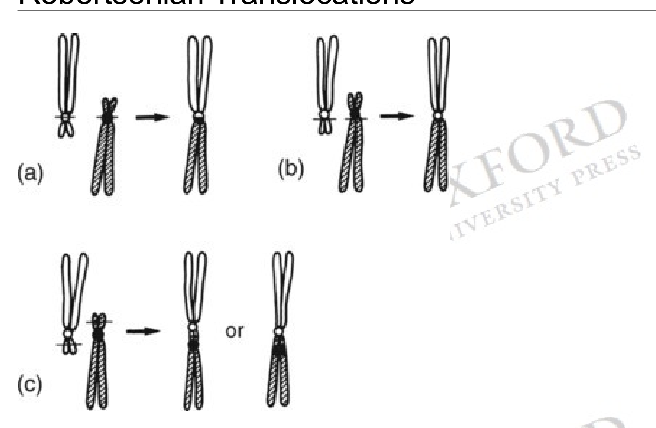




Figure 7–4
Mechanisms of formation of Robertsonian translocations. (*a*) Centric fusion, giving a monocentric chromosome; (*b*) breakage in one short arm and one long arm, giving a monocentric; and (*c*) breakage in both short arms, giving a dicentric or, after suppression of one centromere, a monocentric.

The propensity to recombine may be the consequence of recombination between similar sequences shared by acrocentric chromosomes. The predominance of the rob(13q14q) and the rob(14q21q) may be due to specific homologous but inverted segments in these pairs of chromosomes that encourage crossover, while the variable breakpoints in the uncommon translocations point to a more random process (Bandyopadhyay et al., 2001a, 2001b, 2002). Rare cases are due to postzygotic joining together, a point that can be proven when the two component chromosomes can be shown to have come one from one parent, and one from the other (Bandyopadhyay et al., 2003). Just as a Robertsonian translocation can form de novo from the fusion of chromosomes, so can it (very rarely) revert to two separate chromosomes by a "back-mutational" fission (Pflueger et al., 1991) (and see p. [link]).

The balanced *homologous* Robertsonian chromosome may arise from fusion in the zygote of the paternal and maternal homologs, in which case it is a true translocation. The site of formation may be at the first mitosis, a conclusion we drew from studying a woman with 45,XX,rob(13q13q), who showed no mosaicism on biopsy samples from a number of different tissues taken during surgery for tubal ligation (Gardner et al., 1974). Alternatively, it may be an isochromosome, with the stage having been set in meiosis: a nullisomic egg due to a maternal nondisjunction leads to a monosomic conceptus, which is then "rescued" by reduplication of the paternal homolog as an isochromosome, and thus with uniparental disomy for the chromosome in question (discussed later). Berend et al. (1999) showed a de novo 45,i(13q),upd(13)pat in a normal infant to have complete isozygosity for chromosome 13 markers, indicative of this scenario of postzygotic monosomy rescue. In another instance, they could show a paternal meiotic origin of the i(13q) in a normal adolescent with 45,i(13q),upd(13)pat. This individual would have had trisomy 13, had it not been for gametic complementation: the mother contributed a nullisomic 13 ovum (she being, by extraordinary coincidence, a 13q14q heterozygote). These two cases came to attention only through fortuitous discovery at prenatal diagnosis.

Nucleolar Organizing Regions and the Robertsonian Translocation.

The nucleolar organizing regions (NORs) are located in the "stalks" of the short arms of the acrocentric chromosomes, in the p12 regions, and comprise multiple copies of genes coding for ribosomal RNA. Not all NORs are active: as judged by silver (Ag-NOR) staining, most individuals have four to seven per cell that are functioning (Varley, 1977). Presumably, there is a minimum requirement for normal cellular function. When a Robertsonian translocation forms, the NORs of two of the fusing chromosomes are lost, at least with the rob(13q14q) and rob(14q21q). Thus, an individual with a Robertsonian translocation has only eight acrocentric short arms and therefore eight NORs, but this is nevertheless a sufficient number.

The Heterologous Robertsonian Translocation

Details of Meiotic Behavior

This type of Robertsonian translocation chromosome comprises the long arm elements of two *different* acrocentric chromosomes. At meiosis in the heterozygote, the translocation chromosome and the two normal acrocentric homologs synapse as a trivalent. Following 2:1 segregation, six types of gamete are produced (Fig. **7–5**). "Alternate" segregation leads to the production of normal and balanced gametes; and adjacent segregation produces two types of disomic and two types of nullisomic gamete. 3:0 segregation occurs, but it is rare. In obvious contrast to what happens with the reciprocal translocation, the chromosomally abnormal conceptuses have a complete aneuploidy. Only unbalanced conceptuses that are effectively trisomic for chromosome 13 or 21 can survive substantially through the course of the pregnancy (whether to fetal death in utero, stillbirth, or live birth). Fetal trisomy 14, 15, and 22 are expected to end in miscarriage in the first or early second trimester; and any of the monosomies would abort in the very early first trimester, possibly before the time of implantation.

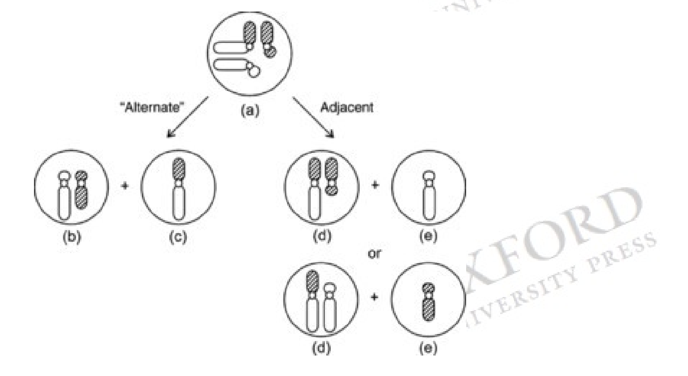




Figure 7–5
Meiotic behavior of the Robertsonian translocation. (*a*) Trivalent at synapsis. (*b*) Normal and (*c*) carrier gametes from *"alternate"* segregation. (*d*) Disomic and (*e*) nullisomic gametes from *adjacent* segregation. Note that there are six possible combinations (ignoring 3:0 segregation), of which two are normal/balanced, and four are unbalanced.

Of these six possible outcomes (eight if we include 3:0 segregants), some are more likely to occur than others. Judging from the outcomes at birth, one might conclude that

alternate segregation is favored. From the male heterozygote, translocation Down syndrome (DS) and translocation trisomy 13 are scarcely ever seen in the offspring, and in a fairly small minority for DS, 10%–15%, from the female (Table 7–2). But of course, as just mentioned, there has been the complete selection in utero against some unbalanced forms, and a variable selection against the two potentially viable imbalances, trisomy 13 and trisomy 21.

Table 7–2. Estimates of Risks to Have a Child with Aneuploidy or with a Uniparental Disomy Syndrome, for the Heterologous rob Carrier

	CARRIER PARENT			
	MOTHER		**FATHER**	
rob	**UNBAL.**	**UPD***	**UNBAL.**	**UPD***
13q14q	1%	<½%	<1%	<½%
13q15q	1%	<½%	<1%	<½%
13q21q	10%–15%	–	<1%	–
13q22q	1%	–	<1%	–
14q15q	–	½%	–	<½%
14q21q	10%–15%	<½%	<1%	<½%
14q22q	–	<½%	–	<½%
15q21q	10%–15%	<½%	<1%	<½%
15q22q	–	<½%	–	<½%
21q22q	10%–15%	–	<1%	–

Note: Estimates for the uncommon rob translocations are extrapolated from data for the common robs.

Unbal., unbalanced, with a full aneuploidy for chromosome 13 or 21; UPD, uniparental disomy; UPD*, abnormal child with syndrome of UPD 14 or UPD 15.

Segregation at the level of the gamete is a different story. Sperm and oöcyte studies show considerable fractions of unbalanced forms. (Naturally, most if not all of the individuals proceeding to gamete testing in these reported studies will have experienced reproductive difficulty, and thus the data from their gametes may not necessarily be applicable to the larger number of carriers with apparently normal fertility.) Ogur et al. (2006) have reviewed the literature on sperm analysis and contributed their own cases, and their findings are set out in Table 7–3. Across all types of translocation (and it is likely that no real differences exist), 81%–92% of sperm are normal/balanced due to alternate segregation, and 7%–19% unbalanced due to adjacent segregation. Rounding these figures, 10%–20% of sperm are unbalanced, and 80%–90% are balanced.

Table 7–3. Segregation Patterns in Gamete Studies upon Heterologous Robertsonian Heterozygotes (Several of Whom Had Presented with Infertility)

TRANSLOCATION	SEGREGATION PATTERN	
SPERM	***2:1 ALTERNATE***	***2:1 ADJACENT (fractions with the viable disomy)***
t(13q;14q)	74%–92% (85%)	8%–26% (14%)
t(13q;15q)	76%–93% (82%)	7%–23% (17%)
t(13q;21q)	87%–88% (88%)	11%–12% (11%)
t(13q;22q)	86%	14%
t(14q;15q)	91%–93% (92%)	7%–8% (7%)
t(14q;21q)	72%–93% (87%)	7%–13% (10%)
t(14q;22q)	79%–81% (80%)	19%–20% (19%)
t(15q;22q)	90%	10%
t(21q;22q)	60%–97% (81%)	3%–40% (19%)
Range of the means	81%–92%	7%–19%
OöCYTES	***2:1 ALTERNATE***	***2:1 ADJACENT (fractions with the viable disomy)***
t(13q;14q)[a]	68%	32% (10%)
t(13q;14q)[b]	26%	68% (16%)
t(13q;14q)[c]	60%	20%*
t(13q;14q)[c]	40%	50%*
t(14q;21q)[a]	58%	42% (20%)
t(14q;21q)[b]	43%	57% (0%)
Range	40%–68%	32%–68%

Notes: The numbers of male subjects range from 25 (the common 13;14), to one per translocation type (the 13;22 and 15;22 cases). Each female subject is listed individually, six in all. Just over 24,000 sperm were studied, but only 200 analyzable eggs. Only 2:1 segregants are listed: 3:0 segregants are very rare, in sperm at least (three 3:0 egg segregants are footnoted below*). 2:1 alternate segregants would produce a normal or balanced karyotype in the conceptus; 2:1 adjacent segregants would produce trisomy or monosomy. Figures in parentheses are average ranges (sperm data), and fractions of gametes (oöcyte data), that could have produced the relevant viable aneuploidy, trisomy 13 or trisomy 21, accordingly. See also the review of Bint et al. (2011).

Sperm data: From the review in Ogur et al. 2006, plus 13;15 and 14;22 cases from Moradkhani et al. 2006a and b, and with these other entries: 13;21 (Chen et al., 2007c; Hatakeyama et al., 2006), 13;22 (Anahory et al., 2005), 14;15 (Moradkhani et al., 2006a), 14;22 (Chen et al., 2005d), and 15;22 (Martin et al., 1992).

Oöcyte (polar body) data: From [a]Munné et al. (2000a), [b]Durban et al. (2001), and [c]Pujol et al. (2003). *Shortfall from 100% totals due to three 3:0 forms.

On oöcyte analysis, using the ingenious approach of fluorescence in situ hybridization (FISH) analysis on polar bodies, Munné et al. (2000a) determined in four 45,XX,rob(14q21q) carriers an average 42% of unbalanced forms, and seven 45,XX,rob(13q14q) carriers with an average 32%; others have provided similar data (Table 7–3), and Bint et al (2011) assemble a recent review (and see p. [link]). In the important 14q21q group, about 20% of ova may be disomic 21, and evidently half or more of these are able survive through to term, to give the 10%–15% risk figure for a child with DS, mentioned earlier.

Meiotic Drive.

Meiotic drive is an influence whereby one of the products at meiosis may be favored and have a better than even chance of coming to be in the successful gamete. The Robertsonian translocation provides an apparent example. At the level of the offspring produced, de Villena and Sapienza (2001) demonstrated that children of female carriers of rob translocations have a ratio close to 60:40 for the balanced rob compared to normal karyotypes. No such effect could be confirmed for the male rob carrier. Daniel (2002) has confirmed these interpretations in a retrospective analysis of prenatal diagnosis data, with rigorous attention to the need to avoid bias, showing a 116:81 ratio in favor of balanced carrier offspring compared to normal karyotypes where the mother is the carrier parent, compared to a 42:41 ratio for carrier fathers. At the level of the gamete, Ogur et al. (2006) showed that, among the unbalanced forms in sperm, nullisomies outnumber disomies.

Postzygotic “Correction” and Associated Uniparental Disomy

Trisomic Correction.

An initial translocation trisomy may be “corrected” by mitotic loss of one of the free homologs and lead to uniparental disomy (UPD) in the embryo. For example, a presumed mechanism whereby UPD 15 could arise from a rob(13q15q) parent is outlined in Figure **7–6**. Essentially, adjacent segregation produces a trisomic 15 conception, and then loss of the chromosome 15 contributed from the other parent,[1] at an early postzygotic stage, “corrects” the karyotype. UPD has no untoward effect if the chromosome is not subject to imprinting (except for the question of isozygosity for a recessive gene; see later), and chromosomes 13, 21, and 22 are in this category. If there is UPD for an

imprintable chromosome—in this context, chromosome 14 or 15—a UPD syndrome would result. Engel and Antonarakis (2002) list just four recorded cases from a familial heterologous rob: one of upd(14)mat, one upd(14)pat, and two of upd(15)mat. James et al. (1994) made a specific search in a group of 14 phenotypically abnormal rob carriers, in most of whom the translocation was inherited, and identified only one case of UPD. A similar retrospective study by Berend et al. (2002a) that included 30 phenotypically abnormal heterozygotes with a familial heterologous translocation revealed two patients with UPD, both having a rob(14q15q), one with upd(14)mat, and the other upd(15)pat. Thus, UPD due to a parental rob is extremely rare, with the worldwide total of reported cases barely numbering in double figures. The overall risk to have a child with a UPD syndrome, as determined from prenatal diagnosis data where one parent is a heterologous rob carrier, is 0.8% (see later).

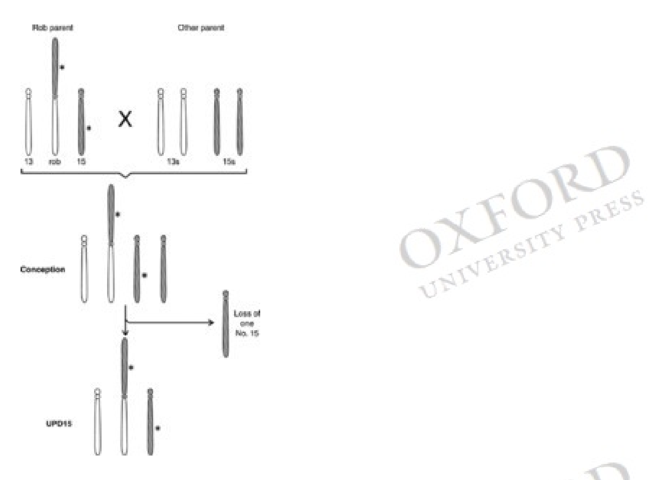




Figure 7–6
Uniparental disomy 15 from a rob(13q15q) parent, due to “trisomy rescue.” The heterozygous parent produces a malsegregant gamete with the translocation, and with a free chromosome 15. The conception thus has trisomy 15. Subsequently, as a postzygotic event, the chromosome 15 from the other parent is lost. Since most malsegregations will have been of maternal origin, the uniparental disomy (UPD) in this setting will usually be a maternal heterodisomy. (Chromosome 13 elements, white; chromosome 15 elements, crosshatched. The two chromosome 15 elements from the carrier parent are asterisked.)

Residual Low-Level Trisomy.

If the “correcting” mitosis occurs too late to include every cell that will contribute to the embryo proper, a translocation trisomic cell line may persist. The only example in the survey of Berend et al. (2000a) was that of a child with upd(13)mat from a rob(13q14q) mother, in whom a low level (4%) of trisomy 13 was shown on prenatal diagnosis, 45,XX,rob(13q14q)[48]/46, XX,+13,rob(13q14q)[2]. Bruyère et al. (2001) record in an abstract three such cases detected in a series of 281 prenatal diagnoses. Jendemy et al. (2010) showed 4%–8% translocation trisomy 13 mosaicism on blood, and 11% on buccal cells, in an abnormal boy whose karyotype could be written 45,XY,rob(13q14q)/46,XY,+13,rob(13q14q)mat; UPD was excluded.

Monosomic Correction.

Hypothetically, correction may also go the other way: that is, the conversion of a monosomic conceptus, due to a nullisomic gamete from 2:1 adjacent segregation in the rob parent, into a disomic conceptus. This conversion to disomy, or “correction,” would be achieved by the replication of the homolog contributed in the (normal) gamete of the other parent. A replicate free chromosome might be produced, in which case the karyotype would appear normal. Or the homolog could replicate as an isochromosome, which would produce the intriguing circumstance of a de novo Robertsonian-like chromosome in the setting of a true Robertsonian parent. This event, whichever one, would take place at a very early postzygotic stage and would necessarily lead to uniparental isodisomy (Berend et al., 2000a). It is, apparently, very rare and might usually fail unless it occurred by day 3, since monosomy becomes a lethal impediment after this stage (Ruggeri et al., 2004).

Association with Infertility

There is an approximately 7-fold excess of Robertsonian heterozygotes among couples who are infertile, and a 13-fold excess among oligospermic men (Tharapel et al., 1985; Ogur et al., 2006). A minority of rob carriers may have an individual predisposition, not necessarily shared by their heterozygous relatives, for a high frequency of unbalanced segregations, an insight that has been afforded by in vitro fertilization (IVF) studies. Alternatively, or additionally, the rob translocation may, of itself, compromise the fidelity of the first few mitoses, affecting mitotic segregation of the other chromosomes (Emiliani et al., 2003). By way of example, Conn et al. (1998) treated two couples with a Robertsonian translocation, who had been unable to achieve a normal pregnancy: a man with 45,rob (13q14q) and a woman with 45,rob(13q21q). They were able to karyotype a total of 33 day-3 embryos from the two couples. A considerable majority of embryos, almost 90%, were chromosomally abnormal. Of these, 40% were trisomic or monosomic for 13, 14, or 21 (some mosaic, and some double monosomic), and this might have been expected. Notably, 60% had a “chaotic karyotype,” in which the chromosome constitution varied randomly from cell to cell, and indeed the karyotype of the original zygote could not usually be determined.

Male Infertility

As noted earlier, there is a 13-fold excess of rob heterozygotes among oligospermic men. A case has been made that, in this setting, synapsis is incomplete in the trivalent, and the heterochromatic regions of the short arms remain unpaired; these “exposed” regions then interfere with pairing in the X-Y bivalent, so that spermatogenesis is blocked from further progression (Luciani et al., 1987; Johannisson et al., 1987). Guichaoua et al. (1990) have directly observed the asynapsed short arms of the trivalent associating with the X-Y bivalent in testicular tissue from an oligospermic man heterozygous for a rob(14q22q), and Navarro et al. (1991) have similarly studied a rob(13q14q) man. Electron microscopic sperm analysis in a rob(14q22q) man with oligoasthenospermia showed marked ultrastructural defects in the great majority of spermatozoa, and attempted IVF was unsuccessful (Baccetti et al., 2002). Mice with several Robertsonian translocations show spermatogenic arrest if the translocations form a chain and associate with the sex chromosomes (Johannisson and Winking, 1994). But it is notable that with some infertile 45,XY, rob(13;14) men, their brothers, fathers, or other male relatives may have unimpaired fertility (Rosenmann et al., 1985).

Rare Complexities

Robertsonian Fission.

The Robertsonian translocations arise through a “fusion” of the short arm sequences. Equally, it can, in somatic tissues, revert to “normality” by fission (Perry et al., 2005). Although the resulting two acrocentric chromosomes would have somewhat truncated short arms that lacked NORs, this appears to be without any clinical consequence (and see also Chapter 8).

Mosaicism for Two Robertsonian Translocations.

A few examples are known of individuals with a 21-containing rob, such as 14q21q or 21q22q, plus an isochromosome of 21q, and presenting as a normal parent of a Down syndrome child, or with mosaic Down syndrome (Gross et al., 1996; Berend et al., 1998; Bandyopadhyay et al., 2003). Iwarsson et al. (2009) undertook sperm analysis in their patient with two Robertsonian translocations, a man whose karyotype of 45,XY, rob(13;13)(q10;q10)/45,XY,rob(13;15)(q10;q10)dn had originally been identified in fetal life, at amniocentesis. Presenting some 22 years later for genetic counseling, a semen analysis revealed oligoasthenoteratozoöspermia, and on FISH study about 40% had disomy or nullisomy 13. These abnormal sperm were presumably due to that fraction of the gonadal tissue bearing the rob(13;13).

Couple Both Heterozygous.

An interesting curiosity is the extremely rare case of a union between Robertsonian heterozygotes. For example, Martinez-Castro et al. (1984) describe two parents both with a 45,rob(13q14q) karyotype, whose three phenotypically normal children had a diploid number of 44, with their chromosomes 13 and 14 existing as a matching pair of rob(13q14q) translocations. Two rob(13q14q) × rob(13q14q) couples, being first cousin pairs and all four having the same rob(13q14q) by descent, each presented with three first-trimester abortions in Bahçe et al. (1996). A couple both carrying a rob(14q21q) are recorded having had a child with Down syndrome, with the unique karyotype 45,XY,rob(14q21q)pat,rob(14q21q)mat,+21mat (Rajangam et al., 1997). Similarly, Mori et al. (1985) reported a couple both of whom were 45,rob (13q15q), and who had had a child with translocation trisomy 13. Due to a founder effect, this otherwise rare Robertsonian translocation was rather common in their small village in the province of Cuidad Real, in Spain, and this couple were surely distantly related, even though they were unaware of any link. The reader may care to construct a hypothetical balanced karyotype with 2n = 41 and five Robertsonian translocation chromosomes.

The Homologous Robertsonian Translocation (Or Acrocentric-Derived Isochromosome)

This Robertsonian translocation chromosome comprises the long arm elements of two acrocentric chromosomes that are the *same*. The site of formation is typically postmeiotic (Robinson et al., 1994). If the translocation forms from the fusion of the two parental homologs, then manifestly there is biparental inheritance (Abrams et al., 2001). If, on the other hand, the rearrangement is actually an isochromosome, each long arm is an exact copy of the other, and there will be uniparental isodisomy. Such an isochromosome may have arisen as a “correction” of monosomy in the one-cell zygote.

Rare cases of mosaicism for a “Robertsonian isochromosome” offer insights into causative mechanisms, albeit that these may not reflect the typical scenario. Bartsch et al. (1993) note some recorded cases of parental mosaicism for 47,+i(21q) and describe their own unique case of a woman with 47,+i(21p)/47,+i(21q)—some hundreds of cells from blood, gonad, marrow, skin were 47,+i(21p), and one single blood cell was 47,+i(21q)—who had had two children with Down syndrome due to the karyotype 46,i(21q). In herself, apparently, the isochromosomes arose as a postzygotic event from a 47,+21 conception, with classic centromere misdivision at the pre-embryo stage. The i(21p) line came to be the predominant in most tissues, but the i(21q) line had at least some representation in gonad and blood.

Details of Meiotic Behavior

Only two segregant outcomes are possible at meiosis in the homologous 45,rob heterozygote. Either the gamete will receive the translocation chromosome, and be effectively disomic, or it will not, and be nullisomic. Essentially, this is 1:0 segregation (or “1+1”:0 segregation). No balanced gamete is possible. Thus, if the other gamete is normal, only trisomic or monosomic conceptions are possible. Occasionally, conceptuses with translocation trisomy 13 are viable, and translocation trisomy 21 not infrequently survives to term. None of the other unbalanced possibilities (trisomies 14, 15, and 22,[2] nor any of the monosomies) are viable.

Postzygotic “trisomic correction” is a mechanism that, rarely, could enable the carrier to have a phenotypically normal child. If, say, in the case of an unbalanced 46,–22,rob(22q22q) conception, the free chromosome 22 were lost at a very early mitosis, genetic balance in this cell line would be restored, with a 45,rob(22q22q) karyotype. Provided the unbalanced cell line contributed negligibly or not at all to the embryo, and provided there were no effect due to uniparental disomy (and in the case of chromosome 22, there is not), the child would be normal. Very few such cases are recorded, with the 13q13q and 22q22q represented (Slater et al., 1994, 1995; Engel and Antonarakis, 2002; Ouldim et al., 2008).

“Monosomic rescue” is another theoretical, and as yet unobserved mechanism in this context, whereby the homolog from the other parent could be duplicated postzygotically, as two separate homologs, or as an isochromosome, to produce a pregnancy with either a normal karyotype, or 45,iso. Finally, for completeness (but almost never in reality), gametic complementation is to be mentioned, whereby the non-rob parent contributes a gamete that happens to lack the homolog for which the rob parent's gamete is disomic (Berend et al., 1999). For the rob(14q14q) and rob(15q15q) carrier, even if one of these rescuing mechanisms did happen, the child would in any event be abnormal, since these UPDs lead of themselves to an abnormal phenotype.

Genetic Counseling

The Heterologous Robertsonian Translocation Carrier

Infertility and Miscarriage

The Robertsonian translocation involving nonhomologs is occasionally associated with repeated spontaneous abortion and with male infertility. The risks for miscarriage are set out in Table 7–4. It may be unclear, in an individual case, whether the association is causal or fortuitous. We can theorize that, in some miscarrying couples, there may have been a majority of zygotes with nonviable adjacent segregants; and in some infertile males, the translocation may have disrupted spermatogenesis. Cytogenetic analysis of products of conception, and of testicular tissue, respectively, may cast some light. It remains possible that some other cause could underlie the problem. The infertile male usually produces some sperm and may thus be a candidate for IVF using intracytoplasmic sperm injection (ICSI) and possibly PGD (Lee and Munné 2000) (and see Table 7–4 and Chapter 26).

Table 7–4. Risk of Miscarriage, and Proportions due to Trisomy, for the 13q14q and 14q21q Robertsonian Translocations, According to Gender of the Carrier

	rob(13q14q)		rob(14q21q)	
	MISCARRIAGE	TRISOMIC	MISCARRIAGE	TRISOMIC
Mother	22%–27%	1%–7%*	24%	10%–14%*
Father	13%	1%	33%	1%

Note: It is apparent that translocation-related trisomy accounts for only a minority of the miscarriages.

* Data depend on the gestational age at the time of ascertainment.

Source: Table adapted from Kim and Shaffer (2002) and Engels et al. (2008).

Risks of Having Abnormal Offspring from Individual Translocations

Figures for the risks to have an abnormal child, or for the probability of detecting an unbalanced form at prenatal diagnosis, are taken (making a few assumptions about extrapolating to the rare translocations) from data of a number of North American and European collaborative studies (Harris et al., 1979; Ferguson-Smith, 1983; Daniel et al., 1989) and set out in Table 7–2. These data relate essentially to the risk for a full trisomy. A potential risk of low-level residual trisomy, perhaps on the order of 1%, might suggest that a good number of amniocytes/colonies be analyzed (Bruyère et al., 2001). Risks for UPD are drawn from the review of Shaffer (2006), again allowing for figures from the more common translocations being applicable to the rarer ones. Detailed comments on each individual translocation follow, with general comments thereafter on the theoretical risks of uniparental disomy, “isozygosity” for a recessive gene, residual low-level trisomy mosaicism, and interchromosomal effect.

The More Common Translocations

rob(13q14q).

The karyotype of the balanced rob(13q14q) is shown in Figure **7–1**. Translocation trisomy 13 can result from adjacent-1 segregation, with a typical Patau syndrome phenotype. The risk for this is very small. Almost all instances are index cases in families, not secondary cases. A review of several pedigrees in Harris et al. (1979), well subjected to statistical rigor, identified *no* increased risk for a malformed infant (they noted that a risk of up to 2% might have been missed, due to the sample size). In a European collaborative study, none of 230 prenatal diagnoses had an unbalanced karyotype (Boué and Gallano 1984), suggesting a risk of less than 0.4%. An incidence in Daniel et al.'s (1989) North American data of 3/204 (1.5%) may have been influenced by ascertainment bias, but in any event, combining the two data sets gives a figure of only 0.7%.

A more recent study, based upon the impressive total of 101 pedigrees, provides support for these historic reports. Engels et al. (2008) identified no cases of translocation trisomy 13, after correction for bias, in live births, albeit that a number of their families had come to attention through an index case with translocation trisomy 13. These authors propose risk estimates of <0.4% for female carriers to have a liveborn child with translocation trisomy 13, and <0.6% for the male, and if the genders are combined, one arrives at a lower figure of <0.23%. They did, however, document a 7% (3/42) incidence in amniocenteses. Further, one translocation trisomy infant had been stillborn; and it is a fine point, in undertaking this sort of analysis, to make a distinction between a stillborn baby versus one that survives only a few days (the usual in Patau syndrome). A risk estimate of ¼%–½%, or more conservatively <1%, may be a practical figure to offer.

If there is male infertility, needing IVF with ICSI to achieve pregnancy, the additional exercise of PGD would be reasonable, to improve the chances of producing a normal/balanced conception; PGD may also be a reasonable choice for some female heterozygotes (see later). Otherwise, an offer of prenatal diagnosis remains a discretionary matter. A focused ultrasound should be capable of detecting the great majority of trisomy 13. The matter of UPD 14 has been noted earlier and discussed again later.

rob(14q21q).

The rob(14q21q) is the most important Robertsonian translocation in terms of its frequency and genetic risk, and it shows a marked difference according to the sex of the parent. Most familial translocation Down syndrome (DS) is due to the rob(14q21q) (Fig. **7–2**). Adjacent segregation may lead to the conception of translocation trisomy 21 (Fig. **7–3**). At amniocentesis, the *female* heterozygote has a risk for translocation trisomy 21 of about 15% (Fergusson-Smith, 1983; Boué and Gallano, 1984; Stene and Stengel-Rutkowski, 1988; Daniel et al., 1989). The risk of having a liveborn child with translocation DS is a little less (in the range 10%–15%): this likely reflects the loss, through spontaneous abortion, of a fraction of DS fetuses after the time during gestation when prenatal diagnosis is done. The risk for the *male* heterozygote is very different, and a figure of <1% is appropriate to offer. The matter of UPD 14 is noted later.

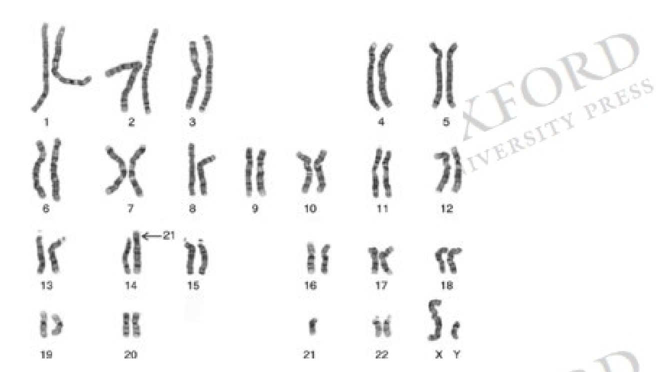




Figure 7–2
The balanced rob(14q21q) in a phenotypically normal male.



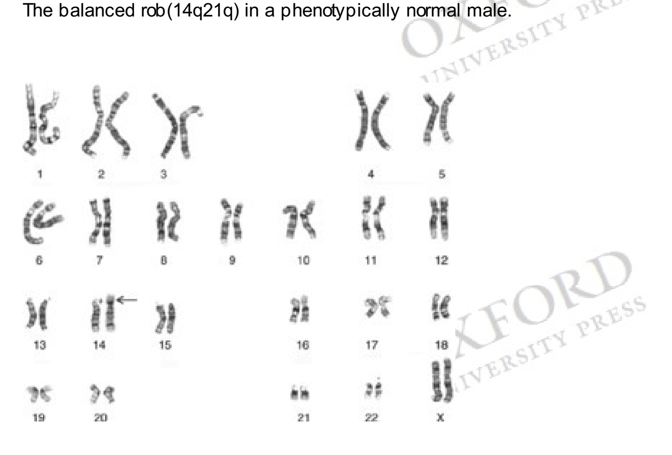




Figure 7–3
The unbalanced rob(14q21q) in a girl with translocation Down syndrome.

The Rare Translocations

rob(13q15q).

Few data are available concerning genetic risks to the carrier (Mori et al., 1985; Daniel et al., 1989). We would expect these individuals are no more likely to produce adjacent segregants than the rob(13q14q) carrier, and a similar risk of <1% for translocation trisomy 13 may therefore apply. The risk for UPD 15 is noted later.

rob(13q21q).

In Boué and Gallano's (1984) study, the risk for translocation DS, in terms of the likelihood of detection at amniocentesis, was 10% for the female; and in Daniel et al.'s (1989) study, the figure was 17%. This 10%–17% range suggests there may be no real difference from the 10%–15% that applies to the common rob(14q21q). The risk for the male heterozygote is low, and probably similar to the <1% proposed for the male rob(14q21q) carrier. A 1% or less risk for translocation trisomy 13 may apply, for either sex. UPD is not a concern.

rob(13q22q).

We presume the risk for translocation trisomy 13 would be "small," and perhaps similar to that for the rob(13q14q); a risk for trisomy 22 would presumably be minuscule.[2] In Boué and Gallano's (1984) study of 262 Robertsonian prenatal diagnoses not involving chromosome 21, there were only 3 rob(13q22q) cases, and in fact one of these showed trisomy 13; no unbalanced karyotypes were diagnosed in Daniel et al.'s (1989) seven cases. The man subjected to a sperm study in Anahory et al. (2005; see Table 7–3) had presented with infertility, and oligospermia was shown. UPD is not an issue.

rob(14q15q).

Adjacent segregants (translocation trisomy 14, translocation trisomy 15) are invariably lethal in utero. UPD 14 or UPD 15 are possible outcomes, as noted later.

rob(14q22q) and rob (15q22q).

The potentially trisomic states from these translocations (trisomy 14, 15, or 22) would all be anticipated to abort spontaneously.[2] Neu et al. (1975) record the segregation of a rob(14q22q) chromosome in a large family, in which some carriers had an increased miscarriage rate. We comment later on UPD.

rob(15q21q).

From Boué and Gallano's (1984) small series of nine carrier mothers, one (11%) had translocation trisomy 21 detected at amniocentesis; and in Daniel et al.'s (1989) data, the fraction was 0/9. These figures derive from too small a body of data to be sure, as yet, that the risk is really any different from the more solidly based 10%–15%, which applies to the rob(14q21q) female carrier. Again, we suppose a low risk (<1%) for the male carrier in terms of DS. The possibility of UPD is noted later.

rob(21q22q).

For a rob(21q22q) carrier parent, the risks for translocation trisomy 21 are about the same as for the rob(14q21q), according to the sex of the parent. UPD need not be a concern, and neither, practically speaking, trisomy 22.[2]

Uniparental Disomy

UPD in a setting of parental Robertsonian heterozygosity is rare. We need consider only those acrocentric chromosomes subject to imprinting: chromosomes 14 and 15. The four syndromes that can theoretically arise are therefore UPD 14, maternal and paternal, and UPD 15, maternal and paternal. The UPD 14 syndromes are described in Engel and Antonarakis (2002) and in Sutton et al. (2003), and maternal and paternal UPD 15 are better known as Prader-Willi and Angelman syndrome, respectively. The potential mechanisms are, as discussed earlier, adjacent segregation followed by "correction" of trisomy with loss of a homolog, or (hypothetically) by "correction" of monosomy with replication of a homolog. In the review of Shaffer (2006), combining prenatal diagnostic data from seven groups, and including both familial and de novo cases, four instances of UPD were identified out of 482 prenatal diagnoses, for a point estimate, therefore, of 0.8%. The two familial cases were upd(13)mat due to a rob(13q14q)mat, and upd(14)mat due to a rob(14q22q)mat; and for the record, the de novo cases were upd(14)mat with a rob(13q14q), and upd(14)mat with a rob(14q21q).

This pooled figure of 0.8% may possibly be a little less in the case of the father being the heterozygous parent (and possibly a little greater in the de novo case), but the numbers are too small to make that call, and it remains quite possible that no such differences exist. For practical purposes, the figure of 0.8% should be seen as applicable across the three parental classes (maternal, paternal, de novo), and across all types of rob. This risk is small, but not negligible. Thus, with respect to the "relevant" robs (those involving chromosome 14 or 15), it may be reasonable to consider adding UPD analysis to karyotyping, if the same 45,rob karyotype as the parent's is observed. As for maternal and paternal UPD 13, 21, and 22, these are, apparently, without phenotypic effect, and need not be a cause for concern.

"Isozygosity" for a Recessive Gene.

Monosomic rescue, whether producing an isochromosome or a 46,N karyotype, theoretically has the potential to cause an autosomal recessive disorder, should the non-rob parent happen to be heterozygous for a mendelian condition the locus for which was on the chromosome in question. But the risk is likely to be very low. In one small series in which a specific search was made for UPD due to monosomic rescue, from a rob parent, no such case came to light, and possibly it may never happen (Ruggeri et al., 2004). Barring knowledge of such a condition (e.g., Bloom syndrome, locus on chromosome 15) elsewhere in the family, molecular testing is not practicable. Of the more common recessive genes that might in some jurisdictions be suitable for population screening (cystic fibrosis, thalassemia, Tay-Sachs disease), none has its locus on an acrocentric chromosome.

Preimplantation Genetic Diagnosis

In the series of Keymolen et al. (2009), a very impressive reproductive improvement was realized with the application of PGD. In 76 rob couples presenting for IVF, and with implantation having occurred (as measured by a positive hCG test), the take-home baby rate thereafter was 71%, compared with only 8% without the use of PGD. Furthermore, success was achieved within about a year, whereas the couples had been trying on average 3½ years before coming to PGD. They had mostly presented due to infertility (male carriers) and recurrent pregnancy loss (female carriers). Emiliani et al. (2003) point out the risk for associated mosaicism of the embryo and advise sampling of two blastomeres, rather than the usual single cell.

Polar body biopsy ("preconception diagnosis") is another approach available through a few IVF clinics, and obviously applicable only to the female heterozygote. Molina Gomes et al. (2009), in a pilot study, describe the procedure in seven women, six with 45,rob(13q14q) and one 45,rob(14q21q).[3] From 32 embryos transferred, three successful pregnancies resulted.

Interchromosomal Effect.

The concept of an interchromosomal effect (ICE) has been invoked in the setting of the balanced Robertsonian heterozygote. Could a translocation somehow influence the distribution of another chromosome not involved in the rearrangement, with the production of a gamete aneuploid for a chromosome not involved in the translocation? Anecdotal reports of DS children born to 14q22q and 13q14q rob carriers (Farag et al., 1987; Sikkema-Raddatz et al., 1997b) seemed to support this notion. However, formal segregation studies in large numbers of families with a rob(13q14q) or with trisomy 21 show no excess of trisomic offspring or of parental Robertsonian translocations, respectively (Harris et al., 1979; Lindenbaum et al., 1985). Therman et al. (1989) ascertained no Robertsonian translocation through a trisomic child other than one that included the trisomic

chromosome. Sperm karyotypes of male heterozygotes show no excess of disomy for other chromosomes (Pellestor, 1990; Syme and Martin, 1992). These pieces of evidence amass a rather strong case that the Robertsonian translocation typically influences the segregation of no chromosomes other than those of which it is comprised. Against these observations, however, are those of Gianaroli et al. (2002), who examined the embryos produced at IVF from a number of rob heterozygotes, eleven male and four female, showing that imbalance due to common aneuploidies (mostly chromosomes 13, 16, 18, 21, 22) actually outnumbered those due to the translocation per se. Their privileged window of observation may have offered a clearer view, although, as indeed these authors acknowledge, the fact of infertility in the couples, and the need in many for ICSI to have been used, may have led to bias; other voices have spoken for this effect (Roux et al., 2005; Ogur et al., 2006; Chen et al., 2007c), and others unconvinced (Munné et al., 2005; Hatakeyama et al., 2006), while Baccetti et al. (2005) reiterate the point that ICE may be a consequence of the compromised spermatogenesis per se. In any event, the propositions that these other chromosomes as listed earlier should be included in the PGD test panel for the rob carrier, and that a case against an ICE is not proven, are to be noted.

The Homologous Robertsonian Translocation Carrier

We refer to these rearrangements as “rob” recognizing, as discussed earlier, that most such cases do actually involve an acrocentric-derived isochromosome (“rob-iso”). Virtually all conceptions of the heterozygote result in either trisomy or monosomy. Monosomy results in occult abortion. Trisomy 14 and 15 always, and trisomy 22 virtually always, miscarry. Most trisomic 13 pregnancies miscarry, although some last until the third trimester; while of course many trisomic 21 pregnancies will proceed through to the birth of a child with Down syndrome. Practically speaking, no normal child could be produced from homologous Rob or isochromosome carrier individuals (the scenario of postzygotic correction, discussed earlier, can scarcely be raised as a realistic hope). Appropriate advice for these carriers is to consider sterilization. Alternatively, the use of donor gametes may allow the couple to have a normal child.

Specific comments relating to the risk for abnormal offspring in each type of rob follow.

rob(13q13q).

The carrier parent can produce only monosomic or trisomic 13 conceptions, and these would either miscarry or, in the case of trisomy, produce a very abnormal child (Patau syndrome). Three recorded exceptions to this statement are given in Slater et al. (1994, 1995) and Stallard et al. (1995), of a normal parent having a normal child with rob(13q13q). The translocations were probably dicentric 13q isochromosomes, arising from postzygotic correction, and thus the children had uniparental isodisomy.[4]

rob(14q14q), rob(15q15q).

Trisomies and monosomies for chromosomes 14 and 15 are not viable, and thus, all pregnancies of these heterozygotes would be expected to terminate in occult abortion or miscarriage. Even if postzygotic correction did happen, the child would have a UPD syndrome, according to the translocation and the sex of the transmitting parent. Thus, it is, in theory and in reality, impossible to have a normal child from any gamete of the heterozygote.

rob(21q21q).

Although the rob(21q21q) is extremely rare, every counselor knows about this famous translocation. It is a classic example of a genetic risk of (practically) 100%. All pregnancies continuing to term can be expected to produce a child with DS. Sudha and Gopinath (1990), for example, report a couple who had 13 pregnancies, with 4 children proven or presumed to have had DS, and 9 miscarriages. The mother was 45,rob(21q21q). No case of postzygotic correction for this translocation has ever been reported.

rob(22q22q).

All conceptions would be monosomic or trisomic 22, other things being equal. For example, one carrier woman had 24 miscarriages, but no normal child (Farah et al., 1975). Two cases are mentioned earlier of postzygotic correction, with the birth of a normal child, but this is not a realistic hope to offer in the individual case.

Prenatal Diagnosis of the De Novo Homologous Robertsonian Translocation

The de novo homologous Robertsonian translocation (or isochromosome) has a high risk for UPD; this entity is commented upon in the chapter on prenatal diagnosis (p. 470).

Notes:

[1] Note that with one or other chromosome 15 being the candidate to be lost, the risk for UPD to be generated is 50%. This is in contrast with correction in standard trisomy, in which, with three candidate chromosomes, the chances are 1 in 3 for the “wrong” one to be lost.

[2] In other settings, trisomy 22, extremely rarely, has gone through to stillbirth, or very short postnatal survival.

[3] It was of interest that in five women where the infertility was due to the male partner, the imbalance rate was 30%, whereas in the two in whom a previous aneuploidy had been documented, the rate was 84%. Further studies will be necessary to confirm whether this finding might be more generally applicable.

[4] This makes the incidental point that UPD 13, maternal or paternal, is without phenotypic effect (Slater et al., 1995).







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Centromere Fissions, Complementary Isochromosomes, Telomeric Fusions, Balancing Supernumerary Chromosomes, and Jumping Translocations

Chapter: Centromere Fissions, Complementary Isochromosomes, Telomeric Fusions, Balancing Supernumerary Chromosomes, and Jumping Translocations
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THIS CHAPTER provides a setting for certain very rare abnormalities that cannot easily be accommodated elsewhere. Barely double-digit numbers, if that, of each category are known. *Centromere fission* results when a metacentric or submetacentric chromosome splits at the centromere, giving rise to two stable telocentric products. In a sense, this is the reverse of what happens in whole arm translocations. The heterozygote, a phenotypically normal individual, thus has 47 chromosomes. With the balanced *complementary isochromosome* carrier, two stable exactly metacentric products are generated. A *balancing small supernumerary marker chromosome* contains material deleted from the normal homolog. *Telomeric fusion* leads to a 45-chromosome count, due to the joining up of two chromosomes, tip to tip, not unlike the Robertsonian mechanism. The fusion chromosome has two centromeres, but one of these becomes inactivated. In *jumping translocations*, a segment can move from one chromosome to two or more recipient chromosomes. The *Robertsonian fission* reverses the fusion that had originally generated it.

Biology

Centromere Fission

In simple terms, a nonacrocentric chromosome undergoes a horizontal splitting at the centromere (Fig. **8–1a**), although the true basis may be more complex than this (Rivera and Cantú, 1986; Perry et al., 2004). Two new telocentric chromosomes result (Fig. **8–2**). One comprises the short arm of the original, and the other its long arm. It is as though the cell ignores the fact that the split happened and continues on normally, treating each part as a properly functioning whole. The other normal homolog remains intact. The heterozygous person (47,cen fis) may have a balanced complement of genetic material and thus be phenotypically normal. Among the few families on record, just six chromosomes—4, 7, 9, 10, 11, and 21—have been involved (Shim et al., 2007). The karyotype may be written, for example, 47,XX,–4,+fis(4)(p10),+fis(4)(q10).

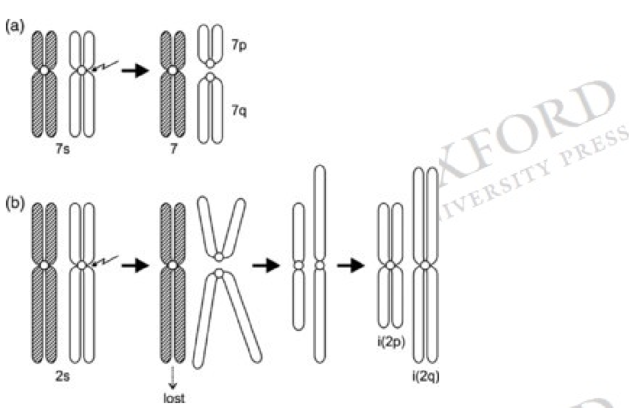




Figure 8–1
Comparing the processes of (*a*) centric fission and (*b*) complementary isochromosomes. The chromosome pairs at the outset (*left*) are to be imagined as existing in the zygote; they have replicated to give the double-chromatid state. The lightning arrow indicates misdivision of the centromere in one homolog. By the time the cell enters the first mitotic division (*right*), the abnormal states have been generated. Note that, according to the proposed mechanism in (*b*), uniparental isodisomy would necessarily result. Open indicates original homolog from one parent; crosshatched indicates original homolog from the other.

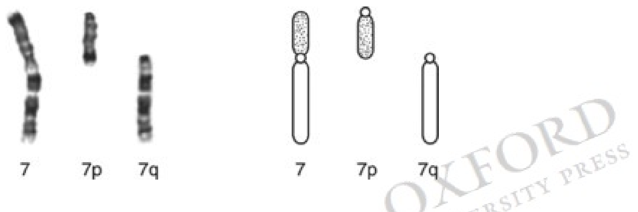


Figure 8–2
Partial karyotype from a case of 47,cen fis(7). One chromosome 7 exists as a normal homolog, and the other homolog is represented by the 7p and the 7q chromosomes.

At meiosis in the heterozygote, the centric fission products presumably form a trivalent with the intact homolog, and 2:1 segregation, essentially as in the Robertsonian carrier, then follows. "Alternate" 2:1 segregation produces normal and balanced centric fission gametes, while adjacent 2:1 segregation leads to gametes disomic or nullisomic for either of the fission products (Fig. **8–3**). Monosomy would probably be associated with occult abortion and trisomy with miscarriage or, in exceptional cases, with the live birth of an abnormal child. Thus far, trisomies for only 4p and 9p are on record.

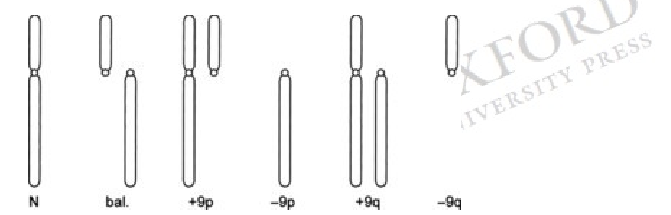


Figure 8–3
The six possible gametes arising from 2:1 segregation in a 47,cen fis(9) heterozygote. Two of these would be normal, the 46,N and the balanced 47,cen fis(9) states. Of the unbalanced states, only the 48,cen fis(9),+9p, in which the imbalance would be a 9p trisomy, might possibly be viable.

The paucity of data does not allow for a precise assessment of the genetic risk run by the centric fission carrier, other than to suggest it could, in some, be quite high. Dallapiccola et al. (1976) reported a chromosome 4 centric fission in a woman who had had two children with trisomy 4p and one normal child. Fryns et al (1980) describe a man and his normal daughter having a centric fission of chromosome 10. Recurrent miscarriage in the families of Janke (1982) and Shim et al. (2007) may well have been a result of asymmetric segregation of a chromosome 7, and a chromosome 11 centric fission, respectively; in the latter case, the cen fis 11 heterozygous woman then went on to have a normal 46,XX child. Miscarriages and childhood deaths in the family of Del Porto et al. (1984) might have been due to a cen fis 4, which was shown to have been transmitted, in balanced state, from a mother to her son.

Extremely Rare Complexity.

A unique case in Fryns et al. (1985) offers a variation on the theme of centromere fission. A mother had deleted an interstitial segment of one of her no. 7 chromosomes, from the centromere to band q21 in the long arm, and this 7(cen-q21) segment existed as a free supernumerary chromosome. The remaining part of 7q, from q21 to qter, joined back on to the original centromere. Since the supernumerary chromosome was mitotically stable, it presumably included functional centromeric material. Her karyotype was balanced. She had had two severely malformed infants who inherited the deleted chromosome, 46,del(7)(cen-q21), but not the supernumerary 7q chromosome, in whom, therefore, the constitutional state was a monosomy for the interstitial segment.

Complementary Isochromosomes

A not dissimilar case is the balanced isochromosome carrier: the "complementary short arm and long arm (p, q) isochromosome heterozygote." Chromosomes 1, 2, 4, 7, and 9 have been reported with this picture, and at least four instances are known for chromosome 2 (Bernasconi et al., 1996; Shaffer et al., 1997; Albrecht et al., 2001; Baumer et al., 2007). The individual has a full complement of the chromosomal material—and may thus be phenotypically normal—but with the two p arms combined in one chromosome and the two q arms in the other (Fig. **8–4**). A formal karyotype might be written, for example, as 46,XX,i(2)(p10),i(2)(q10).

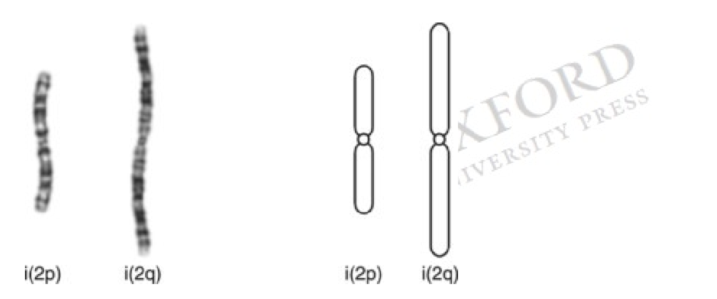


Figure 8–4
Chromosomes from a woman with complementary isochromosomes i(2p) and i(2q) (and see Fig. **8–1b**). (Case of A. A Schinzel; Bernasconi et al., 1996.)

The usual mechanism of formation may be that, in the zygote, horizontal fission at the centromere of one homologous chromosome produces not two telocentric products (as happened in the fission, mentioned earlier), but two mirror-image metacentric chromosomes: an i(p) and an i(q) chromosome (Fig. **8–1b**). This is followed by segregation of both isochromosomes into one daughter cell. There is loss (if it had ever been there) of the homologous normal chromosome contributed by the other parent (unlike the centric fission, in which the normal homolog is necessarily retained); thus, this is a form of monosomy rescue, which engenders a UPD, usually maternal (Bernasconi et al., 1996; Shaffer et al., 1997b; Björck et al., 1999). In other cases, one isochromosome may be of paternal origin, and the other maternally derived, and this may reflect an initial trisomy rescue followed by postzygotic isochromosome formation (Kotzot et al., 2001; Albrecht et al., 2001; Baumer et al., 2007).

A typical clinical presentation has been multiple miscarriage, in phenotypically normal women (Albrecht et al., 2001). Rather analogous to the rob(21q21q) carrier, it is practically impossible for such a person to have a normal child. Any pregnancies from "symmetric" segregation would be either dup(p)/del(q), or dup(q)/del(p), and thus hugely imbalanced.

Balancing Supernumerary Chromosomes

If deleted material from a chromosome is then accommodated in a newly formed small supernumerary marker chromosome (sSMC), and if this extra chromosome can be stably transmitted, then the carrier individual can be of normal phenotype but may have a risk to have a child with a deletion, or a duplication, of the material in question (Baldwin et al., 2008, and see p. [link]).

The most remarkable example is that of a four-generation family, in which several persons carried a chromosome 22 with a classical q11 deletion, but this in company with a small supernumerary ring chromosome that comprised the deleted 22q11 material (Nevado et al., 2009). These people had, therefore, a balanced karyotype, and were phenotypically normal: 47,del(22)(q11),+sSMC. On classical karyotyping, the two chromosome 22 homologs had appeared normal, and it required fluorescence in situ hybridization (FISH) to reveal the deletion on one homolog; thus, the initial impression in this scenario may simply be 47,+sSMC, and the sSMC interpreted as “harmless.” In fact, two of these family members had had a child with typical deletion 22q11 syndrome. The other potential imbalance, that of dup(22q11) due to a 47,+sSMC karyotype, had not been observed in the family.

Telomeric Fusion

This is the tip-to-tip fusion of two complete, or practically complete, chromosomes, and the person thus has a 45-chromosome count (Engelen et al., 2000; Lemyre et al., 2001). The fusion occurs at the level of the telomere or the subtelomeric region. All the necessary functional genetic material is “present and correct” (if there is a missing bit, it contains no crucial genes), and the phenotype is normal, other things being equal. The composite chromosome has two centromeres (hence an alternative name of “stable non-Robertsonian dicentric chromosome”), but one of the two centromeres becomes functionally suppressed. The karyotype is written 45,t(A;B) or 45,dic(A;B), where A and B denote the two chromosomes. The short arm of an acrocentric chromosome is very frequently involved, and chromosome 18 is often one of the participating chromosomes. Ascertainment is typically fortuitous, or through reproductive difficulty (recurrent miscarriage, gonadal dysgenesis, oligoteratospermia). Familial transmission is recorded. The attachment of an essentially complete long arm of an acrocentric chromosome to the telomeric region of another autosome is a very similar circumstance (Fig. **8–5**).

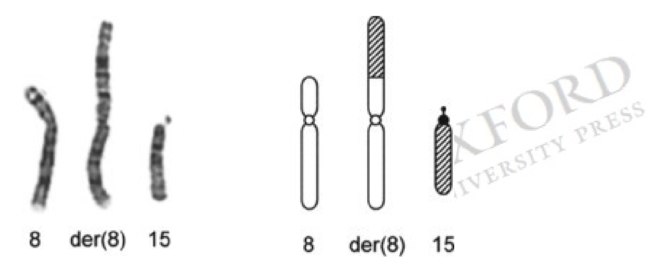


Figure 8–5
A telomeric fusion translocation, 45,XY,t(8;15)(p23.3;q11). The normal father with this karyotype has all the functionally necessary part of chromosome 15 attached to the telomere of a chromosome 8. His child with Angelman syndrome has the same karyotype, but haplotyping with DNA markers showed that both chromosome 15 elements derived from the father, with no chromosome 15 contributed from the mother. Probably, this reflected a “corrected” interchange trisomy. (Case of A. Smith; Smith et al., 1994.)

A normal child could be produced following symmetric, essentially 2:1 segregation: that is, either the two normal homologs are transmitted or the composite chromosome. Asymmetric segregation, were it to happen, would lead to trisomy or monosomy of one of the component chromosomes, and, according to the nature of the chromosome, in utero viability would be compromised. For example, Lemyre et al. (2001) document a 45,XX,dic(14;18)(p11.2;p11.3) mother who was diagnosed, at 32 weeks gestation, with intrauterine fetal death. The fetal pathology examination was consistent with trisomy 18, and the karyotype, 46,XY,+18,dic(14;18) (p11.2;p11.3), confirmed this diagnosis. If the trisomic state were to be “corrected” by loss of the normal homolog from the other parent, a uniparental disomy would result. The case shown in Figure **8–5** is an example of this.

Jumping Translocation (“Translocation Sauteuse”)

This evocative expression describes a mitotic rearrangement whereby the same piece of one chromosome breaks off, on more than one occasion, and attaches to the tips of other chromosomes. The site of breakage in the donor chromosome is characterized by the presence of an interstitial (internal) telomere, and this region offers the possibility of fusion with the recipient chromosomes. Only 26 constitutional cases were listed in the review of Iwarsson et al. (2009).

Levy et al. (2000) identified the phenomenon in two couples, themselves karyotypically normal, presenting with recurrent miscarriage and showing evolving “jumping” cell lines in the cultured products of conception In one of these, for example, the conceptus was initially 46,XX,der(15)t(1;15)(q10;q10). A second line arose, with the 1q part of the der(15) replaced by an additional chromosome 15 which then generated an i(15q), along with (presumably independently) trisomy 7. Five further lines then budded off, all with considerable degrees of imbalance, the pregnancy eventually terminating in first-trimester abortion. Lefort et al. (2001) describe in some detail their own case, an otherwise normal boy with a (possibly coincidental) structural cerebellar defect. He had four separate cell lines, on blood and skin biopsy samples, with the segment 2p12-pter attached to 1pter, 5qter, 6qter, and 12qter, respectively. In each, the rearrangement appeared to be balanced. These authors proposed that these translocations were truly one-way, that is to say, having no reciprocal exchange, and with healing of the 2p12 stump by the formation of new telomeric sequences.

Robertsonian Fission

The Robertsonian translocation is capable of reversing its evolutionary development, and the fused component chromosomes can separate. Perry et al. (2005) studied two families coming to attention due to a known family history of a segregating rob(13;15). They observed fission products, in samplings of somatic tissues (chorionic villus, amniocytes, and blood) in 11 individuals or pregnancies, although mostly at single-digit percentage levels. These “new” acrocentric chromosomes were actually telocentric chromosomes 13 and 15, having no visible short arm material. This phenomenon appeared to be without any clinical consequence.

Genetic counseling

Centromere Fission

The centric fission heterozygote has a significant risk of having a phenotypically abnormal child in those cases in which a whole arm aneuploidy is viable. The 4p and 9p trisomies are the only examples known so far. It is most unlikely that any combination other than the short arm trisomy could be viable. Five percent to 25% is an educated guess of the likely risk range. Prenatal testing is certainly advisable. Of the phenotypically normal offspring of the heterozygote, half would be expected to have the centric fission and half to have normal chromosomes. For the heterozygote in whom neither whole arm imbalance is viable—an obvious example would be a 47,cen fis(1)—no risk for a liveborn abnormal child exists.

Complementary Isochromosomes

In contrast, the carrier of the complementary p/q isochromosome carrier, essentially with certainty (that is, barring an extraordinary rescue event), cannot have a normal child.

Balancing Small Supernumerary Marker Chromosome

The genetic risk is high, and it may approach 50%, if the del or dup imbalance implied by the material contained in the small supernumerary marker chromosome (sSMC) is "genetically small." Nevado et al. (2009) emphasize the need to seek a cryptic deletion in persons found to carry a sSMC; if the true state of a cryptic deletion is not recognized, genetic advice would be gravely misplaced.

Telomeric Fusion

Infertility may be frequent. If conception is possible, there is likely to be a substantial risk for aneuploidy of one or other of the chromosomes involved in the translocation, but equally, a normal child could be conceived. Uniparental disomy will need to be considered at least in the case of a chromosome 15 being one of the chromosomes.

Jumping Translocation

These cases are typically de novo, and the reason for the chromosome suddenly becoming susceptible in the individual is unknown. The genetic implications for the next generation remain uncertain.

Robertsonian Fission

This appears to be a phenomenon of academic interest, seen only in somatic tissues, and of no clinical consequence.



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Inversions

Chapter: Inversions
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INVERSIONS ARE INTRACHROMOSOMAL structural rearrangements. The commonest is the simple (or single) inversion. If the inversion coexists with another rearrangement in the same chromosome, it is a complex inversion. The simple inversion comprises a two-break event involving just one chromosome. The intercalary segment rotates 180°, reinserts, and the breaks unite (Fig. **9–1**). The rearranged chromosome consists of a central inverted segment, and flanking distal, or noninverted segments. If the inverted segment includes the centromere, the inversion is *peri*centric; if it does not, it is *para*centric. Figure **9–2** depicts two different pericentric inversions of chromosome 3. Note that the pericentric inversion has one break in the short arm and one in the long arm, whereas in the paracentric inversion both breaks occur in the same arm. Thus, when reading cytogenetic nomenclature, one can readily tell which is which: for example, 46,XX,inv(3)(p25q21) is pericentric and 46,XY,inv(11)(q21q23) is paracentric (inv = inversion). The clinical relevance of inversion chromosomes is that they can set the stage for the generation of recombinant (rec) gametes that may lead to abnormal pregnancy.

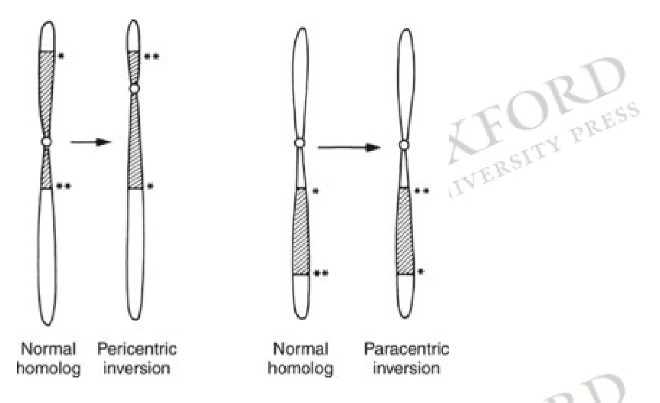


Figure 9–1
The structure of the pericentric (*left*) and paracentric (*right*) inversions. The inverted segment is crosshatched. Asterisks provide landmarks at each end of the inversion segment.

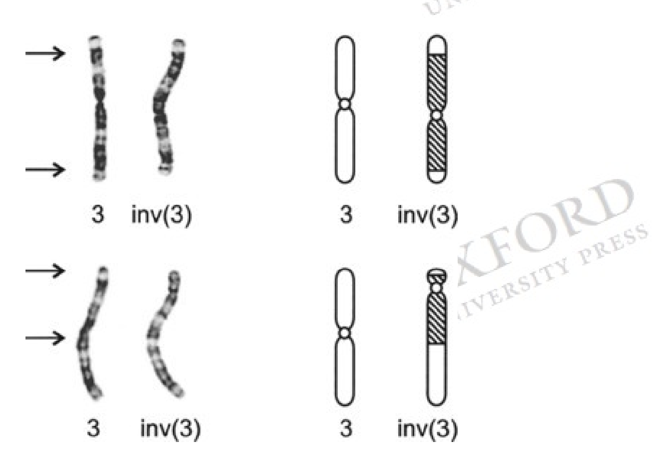




Figure 9–2

Two pericentric inversions of chromosome 3. Both of the noninverted segments are small in one (*a*) and one is large in the other (*b*). (Cases of N. A. Monk and L. M. Columbano-Green.)

The heterozygote is, other things being equal, a phenotypically normal person. The reorientation of a sequence of genetic material apparently does not influence its function, and breakage and reunion at most sites do not perturb the smooth running of the genome. Some inversions of the X may be an exception to this rule: a breakpoint involving the X long arm within the "critical region" can cause gonadal insufficiency. Some pericentric breakpoints occur at preferential sites, including 2p13, 2q21, 5q13, 5q31, 6q21, 10q22, and 12q13 (Kleczkowska et al., 1987); and certain paracentric breakpoints are likewise overrepresented (Madan, 1995).

Cryptic inversions

An inversion may not necessarily be detected on routine study, and knowing when to mount a directed search requires clinical acumen. Thus, Yokoyama et al. (1997) discovered an inv(17) (p13.1q25.1) in a father whose child had lissencephaly, a particular type of severe brain malformation. At first sight, the inverted chromosome looked normal. They noted a family history of similarly affected children, suspected a diagnosis of Miller-Dieker syndrome (which is due to 17p13.3 deletion), and went on to demonstrate the cytogenetic abnormality using fluorescence in situ hybridization (FISH) with a Miller-Dieker chromosome 17 cosmid probe.

Chia et al. (2001) studied a girl with an apparent del(2)(q37) on high-resolution analysis. Using subtelomeric probes to clarify the nature of the deletion, they were surprised to see a 2p signal at each end of the chromosome. Thus, the "deletion chromosome" could be seen for what it really was: a recombinant inversion chromosome, the essential genetic consequence of which was a deficiency of distal 2q. Since the short arm breakpoint was right at the tip of the chromosome at 2p25.3, there may have been little or no duplication of functional 2p genetic material.

Microarray methodology was needed to clarify in detail the nature of a chromosome 20 inversion, in which classical karyotyping had been interpreted as normal, but with multiplex ligation-dependent amplification (MLPA) and FISH then revealing a del/dup of distal 20p/20q, respectively, in three adult siblings (Stevens et al., 2009). The mother's karyotype was 46,XX,inv(20)(p13q13.33), and the siblings each had the identical rec(20)dup(20q)inv(20)(p13q13.33)mat. The imbalances were molecularly very small, the duplication being 2.5 Mb, and the deletion, 1.1 Mb. Dysmorphology was subtle, but the cognitive/behavioral phenotypes were quite abnormal.

Breakpoints within genes

In the event that a breakpoint occurs within a gene, the inversion could be directly pathogenic. Rare de novo examples include an inv(16)(p13.3q13) disrupting the Rubinstein-Taybi syndrome locus, an inv(17)(q12q25) disrupting *SOX9* and causing campomelic syndrome, and a de novo inv(20)(p12.2p13) with one breakpoint occurring between exons 5 and 6 of the *JAG1* gene, causing Alagille syndrome (Maraia et al., 1991; Lacombe et al., 1992; Stankiewicz et al., 2001b). A de novo inv(2)(q35q27.3) provided, in fact, the entrée to the mapping of a Waardenburg syndrome locus to 2q35 (Ishikiryama et al., 1989).

Familial examples include an inv(15)(q11.2q24.3) transmitted from a normal mother to her Angelman syndrome daughter, and which led to the cloning of the *UBE3A* gene, mutation within which causes the syndrome in some subjects (Greger et al., 1997). Potentially, a familial inv(5)(p15.1q11.2) cosegregating with benign neonatal convulsions might reflect a gene disruption (Concolino et al., 2002). In a family with a number of members suffering from attention-deficit disorder, and the affected persons also carrying an inv(3)(p14q21), a locus at each breakpoint was disrupted, these being in an intron of the *DOCK3* gene at the p arm breakpoint, and in an intron of a solute carrier gene (*SLC9A9*) at the q arm (de Silva et al., 2003). One or other of these genes is a fair candidate for having a role in the genesis of this neurobehavioral disorder.

On the X chromosome, a familial inv(X)(p11.4q22) damaging the Norrie syndrome gene is described in Pettenati et al. (1993). Xu et al. (2003) report a family with congenital androgen insensitivity (see p. [link]) segregating an inv(X)(q11.2q27). Presumably, the break at Xq11.2 compromised the integrity of the androgen receptor locus. An inversion chromosome with gene damage at both breakpoints was reported in Saito-Ohara et al. (2002). A mother with the karyotype 46,X,inv(X)(p21.2q22.2) had a severely retarded 46,Y,inv(X)(p21.2q22.2) son with Duchenne muscular dystrophy, these effects being due to disruption of the *dystrophin* gene at Xp21.1, and of the *RLGP* gene at Xq22.2 (and there was also duplication of the proteolipid protein gene at Xq22.2).

Deletion or duplication at inversion breakpoint

A "clean" break and rejoin may not necessarily happen, and the rearrangement may, rarely, comprise, or give rise to, an associated deletion or duplication. Langer-Giedion syndrome (LGS) is due to a deletion at 8q24.11–24.13 (p. 317), and Sasaki et al. (1997) studied a child with LGS who had a de novo inv(8)(q13.1q24.11). Molecular analysis revealed a 4 Mb deletion encompassing the LGS region; presumably this segment had been deleted as part of the process that generated the inversion. A familial inv(18)(q21.1q23), in which a gene for brain myelination and presumably some adjacent genes were deleted, led to some features of the 18q–syndrome in a mother and daughter (Keppler-Noreuil et al., (1998). A familial inv(15)(p11q13), when transmitted from mother to child, underwent loss of the region that contains the putative Angelman syndrome (AS) locus (Webb et al., 1992). The loss was not detectable cytogenetically—the child appeared to have the same inversion that his mother and grandfather carried —but was revealed on molecular analysis. The child had AS. Kähkönen et al. (1990) likewise describe a child with Prader-Willi syndrome and a 15q11 deletion whose father and grandmother were 46,inv(15)(p11q12) carriers.

Lacbawan et al. (1999) reported a child who had inherited a paternal pericentric inversion of chromosome 2 that also had a de novo deletion at the 2p breakpoint, der(2)del(2)(p11.2p13) inv(2)(p11.2q13). The father's inversion 2 is considered to be a normal population variant. The authors reviewed similar cases that occurred on chromosomes 1, 7, 13, 15, and 17 and raise the possibility that the deletions reported were directly related to the presence of the inversion. A deletion may be close to, but not actually a part of, an inversion breakpoint; and this then raises a question of a coincidental association versus a broader effect of the rearrangement (Lybæk et al., 2008).

Inversions in acrocentric chromosomes

Additional complexities may arise when the pericentric inversion involves an acrocentric chromosome, because the nucleolar organizing regions (NORs) become located on the long arm. The first clue that a pericentric inversion is present is the finding of a nonstaining gap in the long arm (this can also be seen if a NOR has been inserted into another chromosome arm). The nonstaining gap can be further characterized/clarified with silver stain, or FISH using a probe to the ribosomal genes. Leach et al. (2005) describe a de novo case, 46,XX,inv(14)(p12q11.2).

"Normal Variant" Inversions.

"Inversions" having a breakpoint within the heterochromatic regions of chromosomes 1, 9, 16, and Y are frequently seen, and they are to be thought of as variants, not abnormal chromosomes (see Chapter 16). The most common inversion in humans not involving centromeric heterochromatin is the inv(2)(p11.2q13); just two recorded cases in the world are known of a *possibly* related pathogenic recombination (see p. [link]). Other presumed harmless inversion variants include the following: inv(3)(p11q11) and inv(3)(p11q12), inv(3)(p13q12), inv(5)(p13q13), and inv(10)(p11.2q21.2). The inv(10) has been rather extensively studied by a collaborative group of five laboratories in the United Kingdom, who had, between them, 33 families available for investigation (Collinson et al., 1997). They found no excess of infertility or spontaneous abortion among carriers; and incidentally, all carriers of the inv(10) may be descendant from the same ancient Northern European heterozygote (Gilling et al., 2006). A similarly large collaborative Canadian study came to a similar conclusion with respect to the inv(2).

Frequency of inversions

Excluding these variant forms, inversions are a fairly uncommonly recognized rearrangement. Estimates of frequency range from about 0.12% to 0.7% (pericentric) and about

0.1% to 0.5% (paracentric) of individuals (Van Dyke et al., 1983; Kleczkowska et al., 1987; Worsham et al., 1989; Pettenati et al., 1995). With respect to the paracentric inversion, Madan (1995), commenting that these are “the most common form of chromosomal polymorphism found in nature,” suspects that many small examples remain undetected.

The Pericentric Inversion Biology

The Autosomal Pericentric Inversion

Details of Meiotic Behavior

The inversion heterozygote may produce chromosomally unbalanced gametes, and in consequence suffer reproductive pathology. The chromosomal imbalance is a result of the formation of a recombinant (rec) chromosome. This is “aneusomie de recombinaison”—aneusomy due to recombination. Recombination occurs if there is, within the inverted segment, a crossover between the inversion chromosome and the normal homolog.

Synapsis and Recombination.

Classically, crossing-over follows the reversed loop model (Figs. **9–3** and **9–4**) (Anton et al., 2005). This configuration of the bivalent allows as complete as possible alignment and pairing of matching segments of the inversion chromosome and its normal homolog (homosynapsis). One (or an uneven number of) crossover(s) within the inversion loop, between a chromatid of the normal homolog and a chromatid of the inversion chromosome, leads to the production of two complementary recombinant chromosomes. One of these has a duplication of the distal segment of the short arm, and a deletion of the distal segment of the long arm (chromosome c-c´ in Fig. **9–4**); and the other way around in the other rec chromosome (d-d´ in Fig. **9–4**). Thus, the conceptuses that result would have both a partial trisomy for one distal segment and a partial monosomy for the other, or vice versa. Typically, only one of these—the least monosomic—is ever viable. Consider the recombinant 7 due to a paternal inversion illustrated in Figure **9–5**. There is a duplication of the substantial segment 7p14.2→pter, and a deletion of only the tiny segment comprising the distalmost sub-band of 7q (7q36.3→qter). The countertype form, having a monosomy for 7p14.2→pter (and trisomy 7q36.3→qter) would, we suppose, cause a miscarriage.

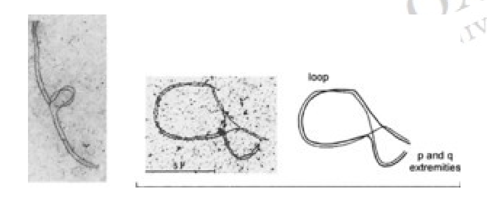


Figure 9–3
Inversion loop in meiosis, direct observation. *Left*, inversion loop in a mouse study. *Right*, spermatocyte study of a man with inv(6)(p22q22.2). (From de Perdigo et al., 1989, Correlation between chromosomal breakpoint positions and synaptic behavior in human males heterozygous for a pericentric inversion, *Human Genetics* 83:274–276. Courtesy Y. Rumpler; reproduced with the permission of Springer-Verlag.)

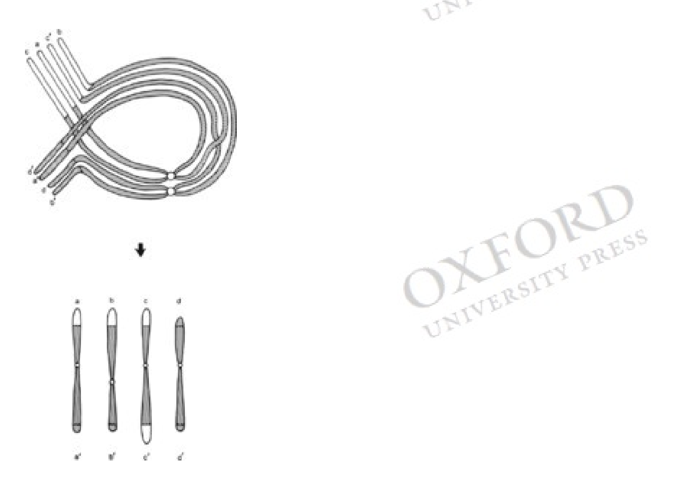


Figure 9–4
Inversion loop in meiosis, theoretical recombinant outcomes (based on the inv(3) shown in Fig. **9–2a**). Both sister chromatids are shown. The inversion (centromeric) segment is crosshatched, the long arm noninverted segment is stippled, and the short arm noninverted segment is open. The four possible gametic outcomes following one crossover within the inversion loop are depicted. Chromosomes a-a´ and b-b´ are the intact homolog and the inversion, respectively; chromosomes c-c´ and d-d´ are the dup p and dup q recombinant chromosomes.

Compare with the actual observation in Figure **9–3**, right.

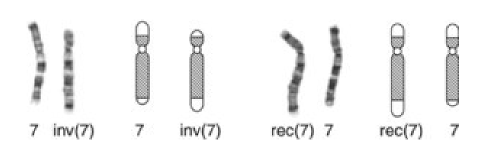


Figure 9–5
Pericentric inversion 7 in father (*left*) of an abnormal child with a recombinant 7 (*right*). The recombinant chromosome has a duplication of just over half of 7p, and a minuscule deletion involving the distalmost subband of 7q. The child has a triple amount of the segment p14.2→pter. The karyotypes are 46,inv(7)(p14.2q36.3) and 46,rec(7)dup(7p)inv(7)(p14.2q36.3)pat. (Case of S. M. White.)

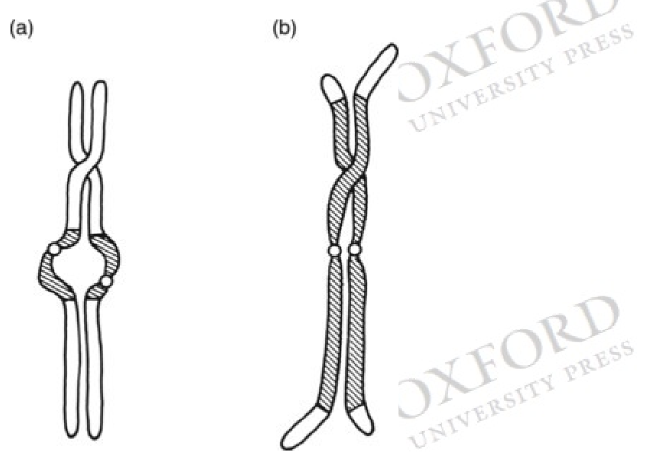
The cytogenetic nomenclature to describe the recombinant karyotype is straightforward. In the above case, for example, we have

Parent: 46,XY,inv(7)(p14.2q36.3)

Recombinant offspring (c-c'): 46,XY,rec(7)dup(7p)inv(7)(p14.2q36.3)

It is not necessary to put 'dup(7p)del(7q)'—the complementary deletion is taken as read. More fully, the nomenclature is 46,XY, rec(7)dup(7p)inv(7)(pter→p14.2::q36.3→p14.2::q36.3→qter)pat.

This complex twisting of the chromosomes to form a loop may not necessarily take place. In an inversion with a short inverted segment (Fig. **9–7a**), a partial pairing may occur. Both distal segments, or sometimes just one, align in homosynapsis. The inverted segment and the corresponding part of the normal homolog either "balloon out" (asynapsis of the inversion segment), or lie adjacent but unmatched (heterosynapsis) (Gabriel-Robez and Rumpler, 1994; Anton et al., 2005). Thus, no crossing-over can happen within the inverted segment, and recombinant products do not form. Conversely, some inversions with long inverted and very short distal segments may undergo synapsis of the inverted segment only, with the distal segments at each end remaining unpaired (Fig. **9–7b**). Recombination can occur in this setting. The quality of the chromatin may of itself have an influence. If both breakpoints are in G-light bands, the lack of homology is detected at synapsis, and the chromosomes respond by formation of a loop, achieving a complete homosynapsis. If, however, one or both breakpoints are in a G-dark band, nonhomology may not be recognized, and heterosynapsis is not prevented (de Perdigo et al., 1989; Ashley, 1990). In this state, recombination is suppressed (Jaarola et al., 1998). With specific reference to some X inversions, it may be that they have a lesser propensity to engage in recombination within the inverted segment (Shashi et al., 1996).



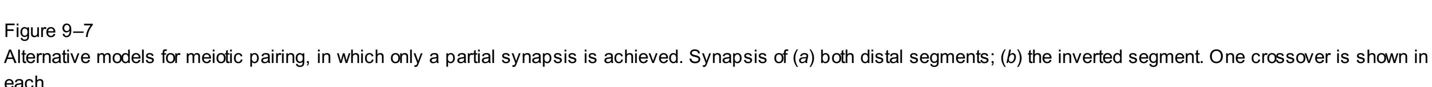


Figure 9–7
Alternative models for meiotic pairing, in which only a partial synapsis is achieved. Synapsis of (*a*) both distal segments; (*b*) the inverted segment. One crossover is shown in each.

Sperm Studies

Sperm studies in a small number of inversion heterozygotes give an indication of the frequency with which recombination happens, at least in male gametogenesis (Anton et al., 2005; Morel et al., 2007). Table 9–2 sets out the findings from a number of such studies. Initially, this work was done using the sperm-hamster methodology; in the late 1990s and 2000s FISH came to be used, and this approach allowed very large numbers of sperm to be analyzed. Dual-color FISH methodology, with one color (say, green) for the p arm and another (say, orange) for the q arm of the inversion chromosome, can show whether a sperm is recombinant. Sperm with nonrecombinant chromosomes would show one orange spot and one green spot. A recombinant chromosome with two orange spots would revealthe dup(q)/del(p) state, while vice versa the dup(p)/del(q) chromosome would have two green spots.

Table 9–2. Sperm Analysis of 22 Autosomal Pericentric Inversion Heterozygotes

			REC		
		INV SEGMENT SIZE (%)	NONRECOMBINANT*	DUP(p)/DEL(q)	DUP(q)/DEL(p)
inv(1)(p31q12)**	30	100	0	0	
inv(1)(p31q12)**	30	99.6	0.25	0.13	
inv(1)(p36q32)	81	83	9	7	
inv(1)(p36.3q43)	95	68	12	19	
inv(2)(p11q13)	10	99.4	0	0	
inv(2)(p11.2q13)	10	100	0	0	
inv(2)(p23q33)	71	61	20	18	
inv(3)(p11q11)	5	100	0	0	
inv(3)(p25q21)	60	69	14	17	
inv(4)(p16q21)	42	99.2	0.8		
inv(6)(p23q25)	80	46	19	19	
inv(7)(p13q36)	65	75	7	17	
inv(8)(p12q21)	31	97	1	0.4	
inv(8)(p12q24.1)	61	61	20	18	
inv(8)(p23q22)**	62	88	6	6	
inv(8)(p23q22)**	62	87	6	7	
inv(9)(p11q13)	16	100	0	0	
inv(10)(p13q22.3)	47	97	3		
inv(12)(p11q23)	51	91	4	4	
inv(17)(p13.1q25.3)	89	73	0.8	0.6	
inv(20)(p12.3q13.33)	84	80	10	8	
inv(20)(p13q11.2)	51	100	0	0	

Notes: Frequencies of recombinant (rec) and nonrecombinant (non-rec) chromosomes are shown as percentages. The size of the inversion segment, as a fraction (%) of the whole chromosome, is noted. Note that, as a rule, the larger the inversion size (especially >50%), the greater the fraction of recombinant forms. The proportions of the two recombinant forms from each inversion chromosome, dup(p)/del(q) and dup(q)/del(p), are very similar.

* Whether normal or the inversion.

** These two pairs represent the same inversion initially studied by the sperm-hamster test, and subsequently by FISH. Note how close the findings are.

Source: From the review of Morel et al. (2007).

The longer the inverted segment, the more likely is recombination to happen. We can separate the studied cases into those with a long inversion segment (over 50% of the length of the whole chromosome), and those in which it is short. In five examples from Table 9–2 with longer inversion segments, inv(3)(p25q21), inv(6)(p23q25), inv(7)(p13q36), inv(8)(p12q24.1), and inv(8)(p23q22), the proportions of dup(p)/del(q) and dup(q)/del(p) recombinant chromosomes were substantial: 31%, 38%, 24%, 38%, and 13%, respectively. No recombinants at all were seen in inversions with a short (or a very short) inversion segment: three “normal variant” pericentromeric inversions of chromosomes 2, 3, and 9, and an inv(20)(p13q11.2). Morel et al. (2007) offer this rule: a high risk of recombination applies when the inversion segment is over 50% in length; the risk is small when the length is between 30% and 50%; and no recombination appears to take place when the inversion segment comprises less than 30% of the chromosome. And in any event, even if recombination occurred in a small inversion segment, the recombinant chromosome would have such a large duplication and deletion that the risk of an abnormal live birth would, very probably, be negligible.

An exception to this rule is given in the long-segment inv(17)(p13.1q25.3) carrier reported in Mikhaail-Philips et al. (2005). Of the 2000 sperm scored, 73% showed balanced segregants, and only 1.4% showed the classical dup/del recombinants. Fifteen percent had deletion only of 17p, which is the basis of Miller-Dieker syndrome (and this the diagnosis in two pregnancies fathered by this man). And similarly, recombination was rare in another inversion with a large inverted segment, inv(1)(p31q12): only 23 recombinants seen in 5966 sperm, a fraction of 0.4% (Jaarola et al., 1998). This reflected a near-complete suppression of recombination.

Different inversions in the same chromosome can have quite different ratios. Caer et al. (2008) looked at sperm from three men, with three different chromosome 8 inversions: p12q21, p12q24.1, and p23q24, respectively. With the p12q21 inversion, almost all sperm, 97%, were nonrecombinant, whereas the other two (with larger inverted segments) had 60% nonrecombinant. Concerning the common inv(2)(p11q13), Ferfouri et al. (2009) studied seven men presenting either with infertility, or during the course of a family study. Of just over 7000 sperm, 99.7% were nonrecombinant; the rate of aneuploidy otherwise did not differ from a control group. This work is interesting in proving that recombination can occur, even with this very short inverted segment; equally, the very tiny fraction manifesting recombination is to be noted.

The fractions of each recombinant type are essentially the same. In the inv(8)(p23q22) listed in Table 9–2, for example, about equal numbers of sperm showed the del(p)/dup(q) state (which is viable), and the dup(p)/del(q) state (which is not), 7% and 6%, respectively.

Segment Content and Viability

While a long inversion segment can set the stage for recombination, what determines the viability of the recombinant conceptus is the functional content of the *noninverted* (distal) segments. We speak of a "genetically small" content if the combined effect of a duplication and deletion does not cause lethality during the earlier part of pregnancy but allows development to proceed well through the pregnancy and possibly to live birth. Thus, only those heterozygotes who have inversions with genetically small distal segments will ever have a chromosomally unbalanced phenotypically abnormal liveborn child. The inversion shown in Figures **9–2a** and **9–5** illustrates this case. Inversion heterozygotes in whom one or both distal segments are genetically large (e.g., Fig. **9–2b**) cannot have an abnormal recombinant child, although they may well have an increased risk for miscarriage. Any recombinants produced by such a person would impart a degree of imbalance that would be lethal in utero.

Genetic content corresponds fairly well to chromosome length. In inversion families in which recombinant children have been born, the distal (noninverted) segments together comprise, on average, only 35% of the total chromosome length; whereas in families having no known recombinant offspring, the figure is 62% (Kaiser, 1988). Nevertheless, if the distal segments comprise "genetically small" material, a larger fraction would not necessarily exclude a reproductive risk. Consider the inv(13)(p11q14) and inv(13)(p12q13), in which the distal segments comprise as much as 75% of the chromosome length. Although the imbalance in the recombinant is large in terms of haploid autosomal length, the result in the dup(q) form is, in effect, a partial trisomy 13 (the partial monosomy for 13p being without phenotypic influence). This is, of course, well known to allow intrauterine and postnatal survival. Similarly, an inversion in chromosome 18 can have distal segments that may be long relative to a short inversion segment, but they are still small genetically, and the dup+del combination can be viable (Schmutz and Pinno, 1986; Ayukawa et al., 1994). With specific reference to chromosome 4, Stipoljev et al. (2002) reviewed 20 reported familial cases and showed that recombinant forms have never been seen in those with smaller inversions, but frequently in the larger ones.

As noted earlier, it is typically the case that only one recombinant form is ever viable. This is rather impressively illustrated in Allderdice et al. (1975) in a kindred with the inv(3)(p25q21). Numerous cases of known or suspected dup(3q) children have been born, but none with the countertype del(3q). There is not even an increase in the miscarriage rate, suggesting that the del(3q) is lethal very early in pregnancy and causes "occult abortion." Viability with both recombinant forms from the same inversion, the dup/del and the reciprocal del/dup, is infrequently seen. Kaiser (1984) records this only in the case of inv(5)(p13q35), inv(13)(p11q22), and inv(18)(p11q21), and Hirsch and Baldinger (1993) add an inv(4)(p15.32q35), as do DeScipio et al. (2010a, b) with respect to an inv(20)(p13q13.3). These five instances have this quality in common: the noninverted segments are very short.

It is instructive to consider the inv(4)(p15.32q35) in Hirsch and Baldinger (1993), in which recombinant offspring could be del(4p)/dup(4q) or dup(4p)/del(4q) (Fig. **9–8**). The four separate segmental imbalances are all well known individually to be viable. Distal 4p is, of course, the basis of the Wolf-Hirschhorn syndrome; and distal 4p trisomy has syndromic, if not eponymic, status. The distal 4q segment is small cytogenetically (0.25% HAL) and functionally, and duplication[1] and deletion are quite well tolerated. So the respective imbalances in the combined states—the del(4p)+dup(4q), and the dup(4p)+del(4q)—remain sufficiently small to be viable, at least much of the time. The index case, with the former imbalance, is a severely retarded child with a Wolf-Hirschhorn phenotype; and an aunt, having the latter combination, had rather minor dysmorphism and mental retardation. The inverted segment is very long: 87% of the total length of chromosome 4. Therefore, a crossover within the inverted segment is, we assume, very likely to take place. Thus, the genetic risks to heterozygotes for this inv(4) is high. Two other reported families, with slightly different breakpoints (4p16.2/4q35.1 and 4p15.1/4q35.1, respectively), also demonstrate a high risk for imbalanced offspring, with both recombinant products observed (Dufke et al., 2000; Maurin et al., 2009).

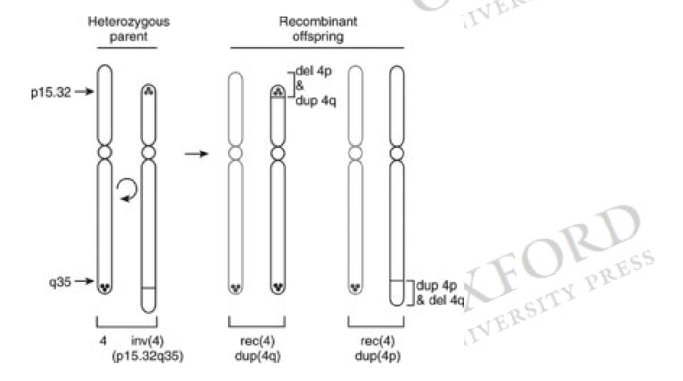




Figure 9–8
An inversion inv(4)(p15.32q35) with small noninverted segments, in which each of the two recombinant possibilities is viable. The del(4p)/dup(4q) karyotype (left recombinant offspring) produces a Wolf-Hirschhorn-like picture, and in the dup(4p)/del(4q) case (right recombinant offspring) the phenotype resembles the partial 4p trisomy syndrome. The normal chromosome 4 contributed by the other parent is shown grayed out. The 4q segment is so small (indicated by the dots) that it might not to make a major contribution, whether duplicated or deleted, to the phenotypes. (From the case of Hirsch and Baldinger, 1993.)

An even higher risk might apply to the inv(13)(p11q22) described in Williamson et al. (1980), in a family with several documented, suspected, or possible recombinant abnormal offspring. Here, the contribution of 13p imbalance to the two recombinant states—the del(13p)+dup(13q) and the dup(13p)+del(13q)—has no phenotypic effect, and the effective "single-segment" imbalances of dup(13)(q22→qter) and del(13)(q22→qter) are each well known to be viable. Applying the principles of "private segregation analysis" set out in Chapter 4, the risk for a recombinant form in this family comes to a high 50%. We emphasize again the point that, while the length of the inverted segment may influence the likelihood of recombination happening, it is actually the combined genetic content of the distal segments that is the direct determinant of viability of the recombinant form.

During the period 1981–1995, over 50 papers were published which reported the birth (or prenatal diagnosis) of offspring having a recombinant chromosome that derived from a parental pericentric inversion. In their review of this body of literature, and adding a family of their own, Ishii et al. (1997) determined the involvement of specific chromosomal segments. Figure **9–6**, which is taken from their paper, depicts the combinations of dup+del genotypes that have been associated with viability. A few of these, which are shown asterisked, were identified at prenatal diagnosis, and in those with no known postnatal case, viability through to term remains unproven. A glance at the figure is enough to see that the gaps—that is, the noninverted segments—are generally longer, and usually a lot longer, than the sum of the lengths of the two inverted segments. This serves to illustrate again the point that inversions with large noninverted segments are, as a rule, the ones with the greatest genetic risk. It is also to be observed that the thick bars (representing duplications) are mostly longer than the thin bars (deletions), a reflection of the preferential viability of the least monosomic combination. The individual autosomal inversions from this review are recorded in Table 9–1.

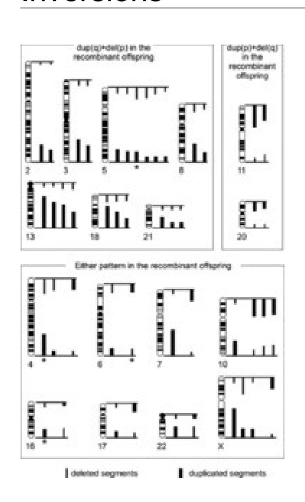






Figure 9–6
Viable recombinants from 55 recorded parental pericentric inversion chromosomes. The pairs of bars alongside each chromosome ideogram, one thick and one thin, show the inverted segments. The thick bars indicate which is the duplicated segment, and the thin bars the deleted segment, in the recombinant offspring. The detail of the actual breakpoints is set out in Table 9–1. The inversions are grouped according to those chromosomes in which a dup(q)+del(p) is consistently seen in the recombinant offspring (*above left*), those in which a dup(p)+del(q) is consistently seen (*above right*), and those in which either pattern may be observed (*below*). Most of these recombinants had been reported in only one or a few cases, with the notable exception of the inv(8)(p23q22), observed on 54 occasions. Asterisks indicate that a case had been diagnosed prenatally; the inv(4)(p13q28) and the inv(5)(p13q33) had been seen only at prenatal diagnosis, so viability to term is not proven in these cases. (From F. Ishii et al., 1997, Case report of rec(7)dup(7q)inv(7)(p22q22) and a review of the recombinants resulting from parental pericentric inversions on any chromosomes, *American Journal of Medical Genetics* 73:290–295. Courtesy F. Ishii; reproduced with the permission of Wiley-Liss.)

Table 9–1. Autosomal Pericentric Inversions from the Literature Review of Ishii et al. (1997) Associated with the Birth of a Recombinant Offspring, Listed in “Numerical” Order

CHROMOSOME		INVERSIONS	
2	p25q35	p25.3q33.3	
3	p25q23	p25q25[a]	
4	p13q28	p15.32q35	
5	p13q33	p13q35	p14q35
	p15q32	p15.1q33.3	p15.1q35.1[a]
	p15.3q35		
6	p23q27	p23.07q25.13	
7	p14.2q36.3	p15q36	p15.1q36
	p22q22		
8	p23q22[a]	p23.3q24.1	
10	p11q26	p11.2q25.2[a]	p12q25
	p15q24		
11	p11q25	p13q23.3	
12	p13q24.3		
13	p11q21	p11q22	p12q13
	p12q14	p13q21[a]	p13q31
16	p13q22	p13.1q22	
17	p11q25	p13.3q25.1	
18	p11q11	p11.2q12.2	p11.2q21.3
19	p13.3q13.33		
20	p11.2q13.3	p12q13.3	p13q13.1
	p13q13.33		
21	p11q21.09	p11.2q22.1	p12q22
22	p11q21	p11.2q13.31	p13q12[a]
	p13q12.2		

Notes: Inversions from 52 families, published over the period 1981–1995, are listed (references in Ishii et al., 1997, plus the case illustrated in Fig. **9–5**; and more recent cases in Lagier-Tourenne et al., 2004, Mehra et al., 2005, Grange et al., 2005, Schluth-Bolard et al., 2008, Tagaya et al., 2008, and Stevens et al., 2009).

[a] Reported in more than one family.

Inversions with very small distal segments may stretch the limits of cytogenetic detection (as also noted in the introductory section). Biesecker et al. (1995) describe an inv(22) with the long arm breakpoint in subtelomeric 22q, with the terminal 23–30 centimorgans of 22q now attached to 22p, which required molecular analysis with microsatellite markers and then FISH with a distal 22q cosmid probe for its identification. Due to the relative lack of G-band landmarks in 22q, and the normal variation that occurs with 22p, the defect was not recognized on a 450-band cytogenetic study. The mother carrying this inversion would have had, presumably, a risk approaching 50% to have a further abnormal recombinant child. Another inv(22) of interest is that described in Boyd et al. (2005), 46,XX,inv(22)(p13q13.1). This rearrangement, in contrast, was very easy to detect, in that the inverted segment involved about half of the long arm and was attached to the particularly long stalk region of the short arm. The stalk region was probably[2] the site of the meiotic recombination which gave rise to her abnormal child with 46,XX,rec(22)dup(22q)inv(22)mat.

Effect upon Fertility.

Uncommonly, the inversion heterozygote can be infertile (Groupe de Cytogénéticiens Français, 1986b; De Braekeleer and Dao, 1991). Abnormal synapsis of the chromosome pair can affect cellular mechanics at meiosis in the male, more likely if the inversion involves a larger chromosome, in consequence arresting spermatogenesis (Gabriel-Robez and Rumpler, 1994). Meschede et al. (1994), for example, describe azoöspermic brothers, one with histologically documented arrest at the level of the primary

spermatocyte, and each heterozygous for an inv(1)(p34q23) inherited from their mother.

Parental Mosaicism.

Mosaicism for a (balanced) inversion is rare indeed. Lazzaro et al. (2001) describe a mother with 46,XX[11]/46,XX,inv(21)(p12q21.1)[19] on blood karyotyping, who had a child with a partial form of Down syndrome. The child's karyotype was nonmosaic 46,XX,rec(21)dup(21q)inv(21)(p12q21.1)mat. Given the mother's karyotype was from a peripheral blood sample, and she having had a recombinant child, clearly enough this is a case of somatic-gonadal mosaicism.

Pericentric Inversions Frequently Innocuous.

Many pericentric inversions are not associated with any discernible reproductive problems. The families of Voiculescu et al. (1986) and Rivas et al. (1987) are not atypical—an inversion chromosome transmitted through several generations, with numerous carriers identified, and no difference between the offspring of carriers, and those of noncarriers, in the incidences of abortion and neonatal death.

Interchromosomal Effect Is Unlikely.

Some pericentric inversions have been discovered in the setting of a child with an aneuploidy such as trisomy 21, and "interchromosomal effect" has been invoked (Groupe de Cytogénéticiens Français, 1985b). More likely, these associations are fortuitous: sperm studies endorse this inference (Anton et al., 2002; Mikhaail-Philips et al., 2004). It is intriguing to note that one case of cystic fibrosis due to maternal uniparental isodisomy 7 occurred in the setting of a maternal pericentric inversion variant for chromosome 7. This link seems more likely coincidental than causal (Voss et al., 1989). In one instance, "intrachromosomal effect" in an inv(21) is considered to have caused trisomic and monosomic 21 conceptions (Gabriel-Robez and Rumpler, 1994).

Rare complexities

Collectors of remarkable cases will find fascinating the report of Allderdice et al. (1991). They studied a kindred (mentioned also earlier) with a segregating inv(3)(p25q21), which originated from a couple marrying in 1817, and which was quite widely spread over the maritime provinces of Canada and other parts of Eastern Canada and the Northeastern United States. In the course of the study, a normal man was found to have two recombinant 3 chromosomes: one with a dup(q)+del(p), and the other with a complementary dup(p)+del(q), such that his karyotype was balanced. Probably, both of his parents were inv(3)(p25q21) heterozygotes, and one produced one recombinant gamete, and the other the other. Essentially the same scenario is described in Kariminejad et al. (2011): a consanguineous couple each heterozygous for inv(18)(p11.31q21.33) produced a child with a complementary recombinant karyotype, of normal phenotype, and in whom analysis showed segmental upd(mat) for 18p and segmental upd(pat) for 18q.[3] A high prevalence of an inversion due to presumed founder effect is proposed in the Guadalajara region of Mexico, from whence there have been reported a number of cases of rec(22)dup(22q) due to a parental inv(22)(p13q12.2) (Tonk et al., 2004).

The Pericentric Inversion X

Pericentric inversions of the X are rare indeed, and in 1997 Madariaga and Rivera were able to review the observations in fewer than 30 families. The X inversion forms in the same way as an autosomal inversion, but the implications are different. This is because *(1)* breakpoints in certain parts of the X (its critical region) may have an influence on the phenotype of the female; *(2)* X chromosomal imbalance in the 46,X,rec(X) female may be mitigated by selective inactivation of the abnormal X; and *(3)* the 46,Y,rec(X) conceptus will have a partial X nullisomy and functional X disomy. The inv(X) can be transmitted both by the males and females. Baumann et al. (1984) and Schorderet et al. (1991), for example, describe families with an inv(X) transmitted through four generations, with all carriers—female heterozygotes and male hemizygotes—being phenotypically normal. The female and male inv(X) carrier need to be looked at separately.

The female inv(X) heterozygote

Outwardly, the female heterozygote appears normal, and not infrequently may be of normal fertility. The concept of "position effect" is of practical importance in the context of X rearrangement. If the long arm breakpoint lies within the segment Xq13→q22 or Xq22→q26, gonadal dysfunction may occur (Therman et al., 1990). There may be primary amenorrhea; or, after a fertile period in early adulthood, a premature menopause. Meiosis would be expected to proceed according to one of the preceding scenarios (see Figs. **9–4** and **9–7**), with recombination within the inverted segment a possibility.

While there is little practical experience to go on, we presume that an ovum with a normal X or the intact (nonrecombinant) inv(X) would produce a normal child, whether male or female. In the case of the male, this would require there to have been no compromise of loci at the breakpoints, and evidence of normality in the male in another family member would be reassuring. A hemizygous son would typically be of normal fertility (Madariaga and Rivera, 1997). If, in the family, the balanced inversion is associated with normal gonadal function in the female, a heterozygous daughter would be expected to have, likewise, normal puberty, fertility, and menopause at the usual time. This family information may not be accessible (or may not exist). In the family of Soler et al. (1981), for example, a hemizygous father had three sons and three daughters—each daughter, of course, an obligate heterozygote. He, apparently, had no gonadal deficiency; but his two older daughters had menopause at 37 and 34 (the youngest was only 30). There was no family history recorded antecedent to him.

An ovum carrying a recombinant X would have two very different results, depending on whether it is fertilized by an X- or a Y-bearing sperm, as follows.

The 46,X,rec(X) Conceptus.

In their review, Madariaga and Rivera (1997) record outcomes in recombinant cases in 10 families. The del(Xq)/dup(Xp) combination is, in female offspring, characterized by normal or tall stature, and ovarian dysgenesis. The countertype, del(Xp)/dup(Xq), is associated with short stature and, in some, intact ovarian function. These phenotypes presumably reflect the loss of stature genes (such as *SHOX*) located on Xp, and ovarian genes located on Xq, respectively. Any effect of the concomitant duplication is, presumably, mitigated by selective inactivation of the recombinant X chromosome. There is no obvious effect upon intellect.

Consider the case presented by Buckton et al. (1981) (Fig. **9–9**). One of the breakpoints is at the tip of the short arm, and the other is in proximal Xq. The recombinant chromosome, with a deficiency of the tip of Xp and a duplication of distal Xq (Fig. **9–9**, lower right), was, in this family, associated only with shortness of stature. The partial Xq trisomy made no discernible contribution to the phenotype. A 26-year-old mother with the rec(X) herself had a rec(X) daughter: unarguable evidence that oögenesis had not (at least by age 26) been compromised.

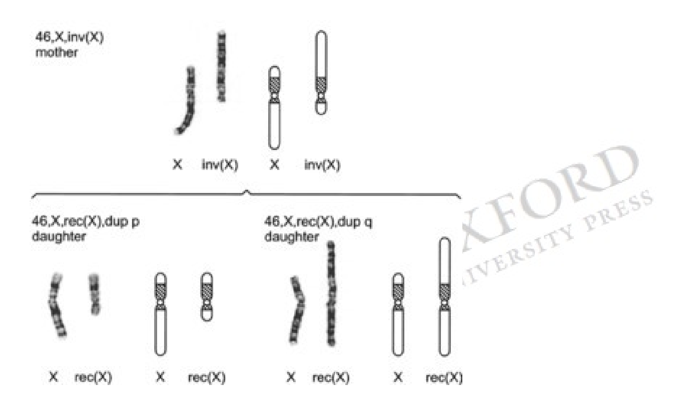




Figure 9–9
X chromosome inversion. The mother (*above*) has the karyotype 46,X, inv(X)(p22q13). *Below*, The two possible unbalanced reproductive outcomes in daughters, following recombination within the inverted segment; the normal X on the left in each has been contributed by the father. Each type of daughter would have a variant form of Turner syndrome. Male recombinant conceptuses are not shown: the combination of X nullisomy and functional X disomy in the 46,Y,rec(X) conceptus would in this instance be lethal in utero. (From the case in Buckton et al., 1981.)

The 46,Y,rec(X) Conceptus.

There will be a nullisomy for the deficient X segment. If this segment constitutes any but the tiniest length of chromatin, the conceptus would not be viable. Nullisomy for a tiny telomeric segment may be viable, but with major dysmorphogenesis and severe neurodevelopmental compromise. Furthermore, the concomitant disomy X is functional, not being subject to inactivation, and therefore of itself produces a major deleterious effect (Groupe de Cytogénéticiens Français, 1986b).

The male inv(X) hemizygote

In the male carrier, the rearrangement apparently has no effect on phenotype or on reproduction. Meiosis proceeds unperturbed (rather obviously, there can be no recombination within the inverted segment). All his daughters will be heterozygotes. Many will have normal gonadal function, although a family history of premature ovarian failure might predict the same problem (see also earlier discussion). Sons receive his normal Y and their mother's (normal) X chromosome.

The Pericentric Inversion Y

A pericentric inversion of the Y,inv(Y)(p11q13), is not uncommon in the general population (Verma et al., 1982; Tóth et al., 1984). It has no phenotypic effect and implies no risk for having an abnormal child. It may be regarded as a normal variant. Meiosis proceeds as it would in the 46,XY male.

Genetic counseling

The Autosomal Pericentric Inversion

Variant Forms

The not uncommon inv(2)(p11.2q13), a very small pericentric inversion, is practically always innocuous (Hysert et al., 2006; Ferfouri et al., 2009). Two possible exceptions are on record to belie its reputation: two abnormal children, one with a 2p duplication and the other a 2p deletion, the proximal boundary at or adjacent to 2p11.2, and the fathers being inversion heterozygotes, described as inv(2)(p11.2q12.2) and inv(2)(p11.2q13), respectively (Magee et al., 1998a; Lacbawan et al., 1999). It may be that the configurations adopted by the chromosome 2 homologs led to an unequal crossing-over, and hence the duplication or deletion. With only two such observations of recombination in the decades of history of clinical cytogenetics, some circumspection is required, and Lacbawan et al.'s comment that “at this point, it seems premature to recommend prenatal diagnosis of all couples in this situation” is reasonable.

No genetic risks are known to be associated with the other inversion variants noted in the “Biology” section: “inversions” of 1, 9, 16, and Y heterochromatin, inv(3)(p11–13q11–12), inv(5)(p13q13), inv(10)(p11.2q21.2). Concerning the inv(10)(p11.2q21.1), Collinson et al. (1997) offer the practical advice that “family investigation of carrier status is not warranted in view of the unnecessary concern this may cause family members.” We exclude these inversion variants from the later discussion.

Risks of having an abnormal child

Ascertainment via Recombinant Child

Identification of a family through a recombinant individual proves the viability of at least one of the two recombinant chromosomes. Table 9–1 lists a large number of different inversions for which a carrier is known to have had a recombinant child. There have been various empiric estimates of the overall level of risk to the heterozygote in families ascertained through an abnormal child. From a number of studies, a consensus range for the usual risk to have a liveborn abnormal child due to recombination is 5%–15% (Sherman et al., 1986; Stene, 1986; Groupe de Cytogénéticiens Français 1986b; Daniel et al., 1989). As a general rule, the longer the inversion segment—and, consequently, the shorter the distal segments—the greater the risk to produce a viable recombinant gamete. Very long inversions, such as that in Roberts et al. (1989), an inv(10) that comprised 80% of the whole chromosome, or the inv(20) in Stevens et al. (2009) comprising 94%, would imply the highest risks: in these particular cases, two out of the inv(10) carrier father's three children were recombinant, and all three of the inv(20) carrier mother's children. For the majority of families, there is probably no risk difference depending on sex of heterozygote (Kaiser, 1984; Stene, 1986); but in some families, the female heterozygote may run a greater genetic risk (Sutherland et al., 1976; Pai et al., 1987). Indeed, for the inv(21)(p12q21.1), recombinant children (with dup(21q) and thus a partial form of Down syndrome) have been seen only where it is the mother who is the carrier parent (Lazzaro et al., 2001).

Each individual inversion carries its own individual risk. This may be arrived at by analyzing the patient's family, studying the literature, and assessing the degrees of imbalance potentially arising in the recombinant conceptuses. A specific figure has been derived for one relatively common inversion, the inv(8)(p23q22): the risk for liveborn recombinant offspring, all of whom would have the del(p)/dup(q) form, is 6.2%, for both maternal and paternal transmission (Smith et al., 1987). This compares closely with the figure of 6.9% of sperm with the del(p)/dup(q) form, attesting to an essentially uncompromised viability of the unbalanced embryo. In contrast, the countertype dup(p)/del(q) recombinant, which is seen in 6.3% of sperm, is never seen in liveborn offspring, reflecting zero viability. With inversions of 18, the breakpoints at p11 and at q11, q12, or q21, a “group risk” of 8% applies (Ayukawa et al., 1994). In due course, figures may be determined for other inversions seen in more than one family, such as the inv(3)(p25q21), inv(4)(p14q35), inv(10)(p11q25), inv(13)(p13q21), and inv(21)(p12q21.1).

The risks to produce abnormal offspring from pericentric inversions in an acrocentric chromosome are again dependent on the size of the inversion, but in this case only the

long arm segment needs to be considered; and rather than a composite del/dup imbalance, a recombinant chromosome would simply convey, in functional essence, either a dup(q), for a partial trisomy, or a del(q), for a partial monosomy. A loss or gain of the p arm material would be without phenotypic consequence. The risk associated with a large inversion, with the q arm breakpoint sited distally, may therefore be particularly high; whereas a small inversion would, as typically, convey the least, and, for chromosomes 14 and 15, a practically zero risk (Leach et al., 2005).

No Family History of Recombinant Form.

For families identified by means other than through the birth of an abnormal child (e.g., discovered fortuitously at prenatal diagnosis), the overall risk is—for what this figure is worth—around 1%. The individual risk, which is what really matters, depends on the actual inversion. Is the inversion chromosome on record (Table 9–1) as being associated with viable imbalance? Or does the inversion segment include and extend beyond the inversion segment of one of these recorded cases? In that circumstance, a significant risk surely does apply (see earlier discussion). Is the inversion segment much shorter in length than any of those listed in Table 9–1? Here, the risk may be as low as zero. The level of risk can be assessed from a study of the family, noting the reproductive histories of other heterozygotes, and from a consideration of the degrees of potential imbalance in a conceptus. As a rule, any chromosome with a short inversion segment (less than one-third of the chromosome's length) is most unlikely ever to lead to a viable recombinant product (Kaiser, 1988; Morel et al., 2007).

Nevertheless, one should determine the composition of the theoretically possible recombinant gametes and gauge whether the resulting partial trisomy and partial monosomy might be viable. This applies in particular to inversions of chromosomes 13, 18, and 21, partial trisomies and partial monosomies of these chromosomes being well recognized as viable. If, in any inversion chromosome, one breakpoint is very close to the telomere, one recombinant form will impose very little partial monosomy. The contribution of the duplication can then be assessed on its own, and reference to the viability of this segment in other cytogenetic contexts (translocation, de novo rearrangement) will likely provide a valid comparison. For example, had the father in Figure **9–5** been identified before he had had children, we could have deduced that the rec(7) dup 7p genotype might survive to term, knowing that the databases of Stene and Stengel-Rutkowski (1988) and Schinzel (2001) record a viable phenotype for trisomy 7p14→pter.

Prenatal (or possibly preimplantation) diagnosis should be offered to the following individuals:

(1) Any heterozygote in whose family a recombinant child has been born
(2) A heterozygote for any of the inversions listed in Table 9–1
(3) A heterozygote for an inversion involving a segment longer than, but including, a region listed in Table 9–1
(4) Any other heterozygote for whom the theoretical recombinant product(s) might be viable. Many inversions of chromosomes 13, 18, and 21 will fall into this category.
(5) Molecular analysis to exclude deletion in the Prader-Willi/Angelman region of 15q11–13 may be appropriate in an inversion having a breakpoint within or adjacent to this segment.

Of the phenotypically normal offspring, approximately half will have normal chromosomes and half will be inversion heterozygotes (Groupe de Cytogénéticiens Français, 1986b).

A risk to the child for some other rearrangement than the classic recombination (see earlier section “Deletion or Duplication at Inversion Breakpoint”) we presume to be very small, likely well under ½%, and prenatal array comparative genome hybridization targeted to the breakpoint regions would not normally be warranted. A question of interchromosomal effect appears not to be an issue.

The Inversion X

The female heterozygote could have a premature menopause, if the long arm breakpoint is in the critical region, and if there is a family history of early ovarian failure; and practical advice might be to have children sooner rather than later. But normal reproductive function is perfectly possible. Recombination may be less likely than for an autosomal inversion (Pinto Leite and Pinto, 2001), although a risk to produce an abnormal daughter with a recombinant X does, nevertheless, exist. The abnormality is, to some extent, predictable according to the deleted segment, Xp or Xq: short stature is typically seen in del(Xp), and ovarian failure in del(Xq). Hemizygous sons would be expected to be normal, and reassurance in this respect may be drawn from the observation, if it can be made, of normality in a male relative. For the most part, no risk exists for having an abnormal son, because recombinant male conceptuses, having partial X nullisomy and disomy, would be nonviable. Only when the breakpoints are very close to the telomere is male viability possible, and such a child would have major physical abnormalities and mental retardation, probably severe. Due to this male lethality, the sex ratio of the offspring would be 1 male:2 females.

All daughters of the male heterozygote would be inv(X) heterozygotes. Other things being equal, they will be phenotypically normal. If the long arm breakpoint is in the “critical region,” and if heterozygous female relatives have had ovarian deficiency (e.g., primary amenorrhea, premature menopause), they may develop the same problem. All sons would have a 46,XY karyotype.

The Inversion Y

This inversion is generally considered a normal population variant of no clinical significance. It is self-evident that all the sons of the inv(Y) carrier will be, themselves, inv(Y) carriers. They are all normal and, other things being equal, have normal gonadal function. All the daughters would be 46,XX.

The Paracentric Inversion Biology

Details of Meiotic Behavior

According to classical theory, the phenotypically normal heterozygote for an *autosomal* paracentric inversion will only have children who are karyotypically normal, or with the same balanced inversion. They cannot have viable unbalanced progeny. If a recombinant gamete is formed following a crossover in the inverted segment, the chromosome would be either acentric (lacking a centromere) or dicentric (Fig. **9–10**). An acentric chromosome is never viable, since it lacks a point of attachment to the spindle fibers. The dicentric is generally considered a lethal impediment, being attached to spindle fibers pulling in opposite directions, with the chromosome thus suspended between the daughter nuclei at telophase and excluded from either cell. If the dicentric were to rupture, however, the possibility theoretically exists for a product (this might be, effectively, a dup+del chromosome) to enter the zygote, and to be viable. Alternatively, if the dicentric were to be included in the nucleus of a gamete, McClintock's classical breakage-fusion-bridge cycle might impose an eventually insuperable obstacle to continuing cell division, as the chromosome is tugged in two directions by its two centromeres in succeeding mitoses after formation of the zygote. The possible scenarios are more fully dealt with in Madan (1995).



Figure 9–10
Theoretical recombinant products from classical crossover in paracentric inversion. One is acentric (ace), and the other dicentric (dic). The inversion segment is shown crosshatched, and the different directions of crosshatching indicate the parts proximal and distal to the crossover point.

What are the findings on direct observation of gametes? In brief, recombination is scarcely ever seen. Anton et al. (2005) review the small total of five sperm studies, with inversion segments ranging from 6% to 32%. The fractions of recombinant sperm ranged from zero to 0.81%. The study with the largest number of cells analyzed (8158) had a recombinant rate of 0.03%, this particular rearrangement described as inv(4)(p14p15.3). The inversion with the largest inverted segment, inv(14)(q24.1q32.1), was analyzed by karyotyping in 120 sperm, and none of them showed a recombinant (Martin, 1999).

Brown et al. (1998) analyzed 282 sperm from a man with a paracentric inversion, 46,inv(9)(q32q34.3), whose wife had had a number of miscarriages (they also had two children). Recombination was suppressed in the inversion segment; but, notably, of the five recombination events within the segment that were observed, each involved at least two crossovers. Brown et al. suggested the following mechanism. Synapsis, which starts at the telomere, advances along the chromatids, and then encounters a region of heterosynapsis and "stalls." This stalling allows an increase in recombination in the chromatid regions that are already synapsed. Synapsis eventually advances past the inversion segment and continues toward the centromere. But within the inversion segment itself, an "active search" for homology goes on, which may require the chromatids to take on a particular configuration (such as a microloop), and this may set up a hotspot for recombination. Only rare double recombinants from this setting would be able to form morphologically normal chromosomes, with sperm that would then be able to continue along their process of maturation; sperm with a single recombination would be acentric or dicentric.

Meiosis in oögenesis commences during fetal life, and its study therefore requires access to fetal tissue. Cheng et al. (1999) analyzed ovarian tissue from a 19-week termination of pregnancy, in which a de novo inv(7)(q11.23q21.2) had been shown at amniocentesis. By using a FISH probe for the Williams syndrome critical region (WSCR), which is at 7q11.23, they could determine whether the inverted segments were aligned alongside each other (homosynapsis) or not (heterosynapsis). Most cells showed the chromosome 7 homologs lined up side by side, but with the WSCR signals off from each other: thus, the inversion segment was unaligned. A classical inversion loop was seen in only 10% of cells. This example, concerning a small inversion segment, offers an explanation for the rarity with which recombinant forms are seen: the necessary prerequisite of homosynapsis may not often be attained.

Recombination/Reunion with Viable Products

Classical theory remains valid in essence, some exceptions notwithstanding. The abnormal process of "U-loop recombination" (Feldman et al., 1993; Mitchell et al., 1994) is a mutational event, not a predictable consequence of a "normal" meiotic process (albeit in a chromosome that is abnormal). *Reunitant* may be a better word than *recombinant*. The crossover within the inversion loop, instead of continuing on in the same direction along the chromatid, reverses upon itself as a "U-loop." The mechanism is illustrated in Figure **9–11**. According to this construction, the resulting reuniting chromosomes would have either a duplication of that part of the inversion loop proximal to the crossover, and a deletion of that part distal to it; or vice versa. A crossover (or, rather, chromatid breakage with abnormal reunion) at one of the entry points to the loop would produce a duplication alone, or a deletion alone.

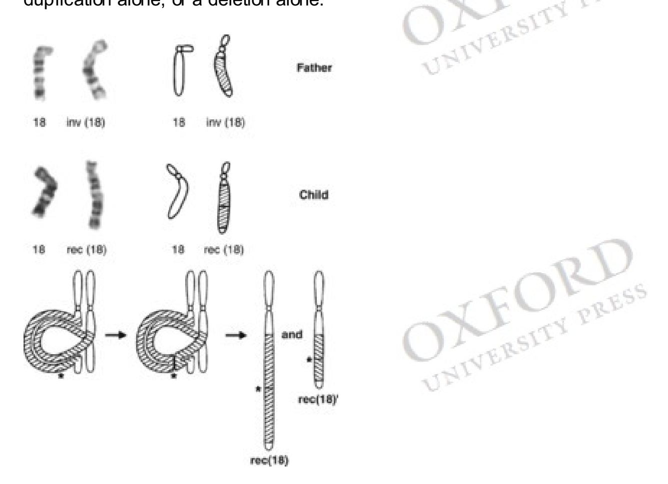




Figure 9–11
Above, parent with paracentric inversion and child with recombinant ("reunitant") chromosome. Father has paracentric inversion of 18q, inv(18)(q12.1q23). The inverted segment is shown crosshatched (crosshatching changes slope at q21.3). Child has duplication of the segment q12.1→21.3 on the reuniting chromosome (shown crosshatched) and deletion q21.3→q23. (Courtesy N. L. Chia and L. R. Bousfield.) *Below*, Proposed mechanism of U-loop exchange depicted; asterisk indicates point of U-loop. The position of the point of exchange within the inversion loop (in this case, q21.3) determines the nature of the imbalance. There is duplication of chromatin proximal to the crossover point (q12.1→q21.3), and deletion of distal chromatin (q21.3→q23), as in the child's rec(18); and vice versa in the complementary product, rec(18)′. (An alternative interpretation is that the father's rearrangement is a within-arm insertion of 18q, rather than an inversion, in which case the karyotype of the child would have been derived from recombination in the inserted segment.)

Feldman et al. (1993) review the inversion duplication (inv dup) chromosome, and notably, of the six familial cases on record, five may have been due to presumed U-type reunion from a maternal paracentric inversion. Chia et al. (1992) describe a case that quite probably reflected the same mechanism, a man with 46,inv(18)(q12.1q23) who had a child with a duplication/deletion 18q syndrome due to a presumed rec(18)(pter→q21.3::q21.3→q12.1::q23→qter) chromosome, as shown in Figure **9–11**. Another very similar

inv(18) case is noted in Hani et al. (1995). In their exhaustive review, Pettenati et al. (1995) collected about a dozen similar cases. These cases represented offspring in 3.8% of their series of 446 paracentric inversions; but since all of these offspring were probands, and some we actually doubt were truly paracentric "reunitants," we presume the actual reproductive risk due to U-loop reunion or other abnormal process would be a much smaller figure (Sutherland et al., 1995). Madan and Nieuwint (2002) pursue this question and show that indeed most "paracentric inversions" found through a recombinant child were really insertions.

Classical theory needs also to accommodate the phenomenon of centromere suppression, which, extremely rarely, can allow the basically dicentric recombinant to function stably as, in effect, a monocentric. The chromosome attaches to the spindle fiber at only one centromere, the other being nonfunctional or suppressed; thus, no fusion-bridge cycle is initiated. "Extremely rarely" could, at this writing, be defined as four recorded cases from an autosomal paracentric inversion. Mules and Stamberg (1984) describe an infant dying as a neonate with a rec(14) whose mother had an inv(14)(q24.2q32.3); Worsham et al. (1989) studied in considerable detail a child with a rec(9) from a maternal inv(9)(q22.1q34.3); Whiteford et al. (2000) report a dysmorphic infant with growth and neurodevelopmental retardation and having a major heart defect, with the karyotype 46,XY,rec(15)(pter-q26.3::q11.2-pter)inv(15)(q11.2q26.3)mat; and Lefort et al. (2002) describe an abnormal child in whose dicentric rec(14) chromosome one centromere could be demonstrated to have been inactivated, the mother's karyotype being 46,XX,inv(14)(q13q32.2). These four cases share the features of a large inversion involving most or almost all of a long arm, and with the short arms (14p, 9p, 15p, and 13p, respectively) being genetically "small": in other words, the dup p+q/del q combination might not impose a lethal imbalance. Only in this setting, and if the dicentric chromosome were stable, could recombination cause an imbalance that would be viable and allow the birth of an abnormal child.

> A mechanism reminiscent of paracentric inversion U-loop reunion may be the cause of some isochromosome Xq Turner syndrome (Wolff et al., 1996). Two zinc-finger genes (*ZXDA* and *ZXDB*) in proximal Xp, just above the centromere, have about 98% homology and transcribe in opposite directions. In X-to-X synapsis in some meioses, a small inversion loop in proximal Xp might enable *ZXDA* (the more centromeric locus) in one Xp to match up with *ZXDB* on the other Xp, and vice versa. Then, a breakage and U-loop reunion between the two ZXD loci would generate an isodicentric chromosome Xqter→cen→*ZXDA*::*ZXDA*→cen→Xqter. Similar events at other loci may underlie other Xq isochromosomes, or supposed Xq isochromosomes (Giglio et al., 2000).

A minuscule number of cases of other sorts of viable recombinant offspring are known (Worsham et al., 1989). A dicentric recombinant chromosome, pulled in two directions, may rupture and yield a deletion. This may be the mechanism in the case in Courtens et al. (1998), in which a mother with 46,XX,inv(18)(q21.1q22.3) had monozygotic twins with a deletion of the segment distal to the inversion (q22.3-qter), and duplication of a small part proximal to it (q12.1-q21.1). In Fig. **9–10**, although the scale is not right for this example, it could be imagined that a break occurred in the dicentric recombinant chromosome just above the lower centromere. An alternative mechanism is that the abnormal synapsis may set up a milieu that encourages some other type of rearrangement to form, such as excision of an inversion loop, and unequal crossing-over at the base of an inversion loop, a format initially proposed by Hoo et al. (1982). Yang et al. (1997) propose such a scenario in a family in which the index child had the deletion 46,XY,del(17)(p11.2p11.2), while the father and two aunts carried the paracentric inversion 46,inv(17)(p11.2p13). The deletion removed the Smith-Magenis region. This was "the first unequivocal demonstration by molecular analysis that a parent who carries a paracentric inversion is capable of having a viable child with an unbalanced monocentric recombinant chromosome." Paskulin et al. (2011) relate a similar story, a child with a deletion in 7q31.32q33 (nt 112,552,898–135,714,528) at the base of the presumed meiotic inversion loop, from a maternal 46,XX,inv(7)(q11.23q33). South et al. (2006) describe a 46,XX,inv(5)(p13.3p15.3) parent who had a child with del(5)(p14.3) cri-du-chat syndrome, which was likely due to a dicentric recombinant chromosome having ruptured in the middle of the inversion segment. Phelan et al. (1993) report the unique case of a father with an inv(9)(p13p24) having a child with a rec(9) containing a tandem duplication, which they propose came from breakage and reunion between sister chromatids within the inversion loop. Another unique case is that described in McClarren et al. (2006), in which an inv parent had a child with a deleted ring 22, leading to diGeorge syndrome. An inversion with a breakpoint in the vicinity of 15q12 may lead to a rearrangement that would cause Prader-Willi syndrome or Angelman syndrome, as mentioned earlier in the pericentric case.

Microarray analysis enables a more precise view of possible recombination/rearrangement from a paracentric inversion, and we have applied this methodology in the laboratory in Spokane to two cases. First, in a child with developmental delay and dysmorphic features, the microarray study showed a single copy gain at 7q31.1q31.31 and a single copy loss at 7q31.33q32.3. The intervening sequence was present in two normal copies. Metaphase and interphase FISH confirmed both the gain and loss. Parental FISH studies were performed and identified a paracentric inversion in the mother. In a second case, we identified a single copy gain at 18q21.32q23. Metaphase FISH confirmed the presence of an additional proximal signal on one chromosome 18. Parental metaphase FISH studies were performed and again revealed an apparently balanced inversion in the mother.

Some inversion carriers have been ascertained through their having had many miscarriages (Madan, 1995). In most of these, surely, the discovery was fortuitous. One family with an inv(10) was widely studied, and 19 carriers in three generations had only one miscarriage out of 36 pregnancies (Venter et al., 1984). The report in Devine et al. (2000) mentioned earlier of two brothers with 46,XY,inv(2)(q14.2q24.3) presenting with reproductive pathology may be suggestive, but other causes are quite possible. One brother's wife had three miscarriages, and at in vitro fertilization in the partner of the other brother, five of ten fertilized eggs failed to cleave, and progression in the remaining five failed at the blastocyst stage. No karyotyping was done of any of these several products of conception. In a very few cases, theoretical dicentric recombinant products might convey a genetic imbalance that could allow at least some weeks of in utero growth before miscarrying (Bocian et al., 1990; Bell et al., 1991). The nine miscarriages suffered by the carrier grandmother in the family in Worsham et al. (1989) we might more reasonably imagine to have been due (some of them at least) to recombinant gametes, the dicentric state having been proven in her index grandchild.

Coincidental Abnormality.

In some instances, the finding of a chromosome abnormality in a child from a paracentric inversion parent may be coincidence, even if the same chromosome is implicated, as seen in the case reported by Bourthoumieu et al. (2003) of a child with cri-du-chat syndrome and a del(5)(p14pter) karyotype. Parental chromosome studies revealed that the mother carried an apparently balanced paracentric inversion of long arm of one chromosome 5. The authors concluded that the two rearrangements were unrelated.

Other Mechanisms Causing Abnormality.

Mendelian loci can be vulnerable when chromosomal rearrangement happens, due to "position effect," epigenetic influence, or direct disruption. We have seen, for example, a family in which a chromosome 7 inversion, inv(7)(p22.2p21.2), has been associated with Saethre-Chotzen syndrome, this being a Mendelian disorder due to the *TWIST* gene. Two heterozygous children showed major craniosynostosis, but the father and grandfather had only the subtlest facial, auricular, and digital signs. It may be that Saethre-Chotzen syndrome due to position effect has a milder phenotype than in the case of point mutation (Rose et al., 1997). On chromosome 7, at 7q21q22, an inversion has been described in association with the split hand/foot malformation, and again it is proposed that "position effect," with compromised expression of a putative hand/foot morphogenesis gene(s) in neighboring chromatin, might be the underlying causal mechanism (van Silfhout et al., 2009). Another possible epigenetic example: Norman et al. (1992) described a family in which a mother had one child with Beckwith syndrome and a presumably affected fetus, all three carrying an apparently balanced inv(11)(p11.2p15.5). The normal imprinting state of the Beckwith region on distal 11p may have been perturbed.

A Special Case, inv8p23.

A paracentric inversion of 8p23 is a very common "abnormality," and indeed should be described as a polymorphism, since it occurs in around a quarter to a third of European and Japanese populations, respectively. The inversion cannot be seen on classic cytogenetics and requires FISH with probes recognizing sequences within band 8p23 for its delineation. From the millions, perhaps billions of people who carry this inversion, the tiniest number of abnormal infants have been born, perhaps 50 or so known worldwide. In these very rare cases, the inversion has led to a classic recombination with production of a dicentric chromosome, essentially as outlined in Figure **9–10**, in which a segment including one centromere is then "clipped off," to produce a monocentric inv dup del(8p)(8qter→8p23::8p23→proximal 8p). The recombinant chromosome is typically generated in (unusually for a structural rearrangement) maternal meiosis (Shimokawa et al., 2004).

X Chromosome.

If a paracentric inv(X) is associated elsewhere in the family with normality, no defect would be anticipated in future heterozygotes or hemizygotes (Neu et al., 1988a). Breakpoints in the critical regions in Xq might, however, compromise gonadal integrity. For example, Dar et al. (1988) report a woman with a de novo inv(X)(q13q24) who had ovarian dysgenesis with primary amenorrhea and no spontaneous pubertal development, and Németh et al. (2002) describe an infertile man with a Klinefelter-like phenotype having an X inversion with rather similar breakpoints, 46,Y,inv(X)(q12q25). A woman with a somatotype of Turner syndrome having an Xp inversion (p11.2p22.1) is described in Dahoun (1990). A breakpoint might damage a mendelian locus, and Briault et al. (1999) report a family in which the FG syndrome (mental defect, facial dysmorphism, hypotonia, anal abnormality) cosegregated with a paracentric X inversion, inv(X)(q12q28). One FG locus has been mapped to Xq12-q21.31, and so it is plausible that the q12 breakpoint in the inversion may have been at the site of the FG gene.

Y Chromosome.

Only two paracentric inv(Yq) cases are on record (Madan, 1995; Liou et al., 1997). In Liou et al.'s three-generation family, the normal grandfather and father were 46,X,inv(Y)(q11q21), and the child with the same karyotype had ambiguous external genitalia with Müllerian structures internally and intra-abdominal testes. The inversion Y may have been coincidental; alternatively, there may have been, in the child, a position effect whereby the expression of a gonadogenesis gene had been compromised.

Paracentric inversions usually innocuous

The above rather extensive compendium notwithstanding, the observed facts attest to the general innocuousness of the autosomal paracentric inversion, concerning either the heterozygous state per se, or a risk for chromosomally unbalanced offspring. Madan (1995) reviewed 184 cases of autosomal paracentric heterozygosity. Many were ascertained fortuitously, and including those discovered during the course of investigation for recurrent miscarriage, 58% were identified in a normal person. Several had an abnormal phenotype, but this was, of course, the reason they had the chromosome test done in the first place: by definition, they had to be abnormal. No clear consistent pattern among phenotypes of presenting cases is apparent. As Madan comments, there may have been a bias in choosing cases for publication, and editors of journals might not find compelling a paper describing an “uninteresting” inversion discovered incidentally in a normal individual (a series of a dozen or so cases might stand a better chance). In their review, Pettenati et al. (1995) could be confident about a causal association with a specific phenotype only in the paracentric inv(X), and not with any of the autosomal inversions.

The Groupe de Cytogénéticiens Français (1986a) note that the reproductive fitness of heterozygotes in 32 French families was normal. Two quite common inversions seen in a number of families in more than one part of the world are the inv(3)(p13p25) and the inv(11)(q21q23) (Madan, 1995). No abnormalities directly attributable to these inversions have been documented. It may be founder effect, or recurring mutation, that is the basis for their frequency. In the one sperm study of a paracentric inversion heterozygote, having the relatively common inv(7)(q11q22), Martin (1986) found no recombinants. The smallness of the inversion segment may have been a factor militating against formation of a synaptic loop. (It is not without interest to note that a similar inversion is the norm in the gorilla chromosome 7, and so this human form could be thought of as a “back mutation” to that of the ancestral primate.)

Interchromosomal Effect Is Unlikely.

Watt et al. (1986) raise the possibility that the paracentric inversion might have an “interchromosomal effect.” They note an apparently high level of reported associations, within families, of an inversion plus some other chromosomal defect. We suspect this is artifactual; as these authors note, ascertainment and publication biases are potential confounders in this setting. Pettenati et al. (1995) reached a similar conclusion.

Technical Comment.

Paracentric inversions can be technically difficult to detect on classical cytogenetics. Gross chromosome morphology is not altered, and unless major landmark bands are shifted, the rearrangement may go unnoticed. Only with the use of good quality, high-resolution banding are paracentric inversions likely to be detected regularly. These cytogenetic difficulties may be why relatively few cases of this type of inversion have been published. Also, for technical reasons, reported cases of recombination in the literature should be regarded with caution; as mentioned earlier and noted later, some “inversions” are likely actually to be intrachromosomal insertions (“paracentric shifts”). The cytogenetic distinction can be difficult to make, especially so for chromosomal regions without distinctive banding patterns, or where the inverted segments are very small (Callen et al., 1985; Madan, 1995). For example, the inverted insertion of chromosome 15 described in Collinson et al. (2004), associated with recombinant offspring having Prader-Willi and Angelman syndrome (and see p. [link]), had originally been reported, some 10 years prior, as a paracentric inversion. We have seen a family in which the index case seemed to have an unbalanced translocation at distal 4p, but the normal mother and grandfather had the same anomaly, which could then be reinterpreted as the minimum inversion detectable on routine cytogenetics, a one-band paracentric inversion, in this case inv(4)(p15.3p16.3) (Smith et al., 1992).

Genetic counseling

On practical grounds, the reassuring point to note is that practically all paracentric inversion heterozygotes identified have been discovered fortuitously, and not through the birth of a child with an abnormality attributable to the parental inversion (Madan, 1995). We agree with Madan: “the vast majority of paracentric inversions are likely to be harmless.” Apparently, the genetic risks to offspring are extremely small. In the U.S. collaborative study described in Daniel et al. (1988), there were no unbalanced karyotypes in 30 prenatal diagnoses. The sex chromosomes warrant separate attention, and it may be that some X and Y paracentric inversions have an effect upon gonadal development in the intact (that is, unrecombined) state.

However, a tiny handful of abnormal offspring, and as reviewed at length earlier, refute a complete harmlessness in the parental paracentric inversion, whether due to classic recombination or to other forms of rearrangement. Whether this would warrant prenatal diagnosis, when a parent is a carrier of one of these inversions, or indeed for any paracentric inversion, is a matter for debate. Even where the new chromosome from a classic recombinant or U-loop reunion might on theoretical grounds be viable, the risk for one to be generated, while its exact magnitude is unknown, is surely “extremely small.” “Better than 99.9%” might be a fair estimate that there will be no untoward reproductive outcome due to behavior of the inversion. Albeit that microarray may offer the potential to screen, at prenatal diagnosis, for submicroscopic molecular damage associated with a particular apparently balanced inversion, the case for so doing is very modest. Nevertheless, it would scarcely be realistic, at the present time (although the future may hold a different scenario), to screen, at prenatal diagnosis, for submicroscopic molecular damage associated with a particular apparently balanced inversion. Caution should be exercised during genetic counseling, in that it is prudent never to say “never,” and no risks are ever zero.

Thus, we suggest that, in practice, an offer of prenatal diagnosis be discretionary, in the case of a fortuitously discovered inversion in the family; and we would regard it as not inappropriate if the offer were declined. A firmer stance may be appropriate if there has been a previous history of an apparently associated reproductive abnormality. Inversions on record with a demonstrated recombinant would oblige the offer of prenatal diagnosis. These include those noted earlier: inv (7)(q31.31q31.33), inv(9)(p13p24), inv(9)(q22.1q34.3), inv(14)(q24.2q32.3), inv(17)(p11.2p13), inv(18)(q12.1q23), inv(18) (q21.1q22.3), and inv(18)(q21.32q23). But again, we return to the expressions used above: “vast majority”, and “better than 99.9%”, in respect of favorable behavior of the inversion.

As mentioned earlier, a diagnosis of a paracentric inversion might be incorrect, and the rearrangement is actually a within-arm insertion, which carries a high genetic risk (p. 194). Since the distinction in the routine laboratory can be difficult, a practical view might be to risk overinterpreting subtle paracentric inversions as potential insertions, in those cases where the cytogeneticist is not absolutely certain. The true picture may emerge by determining the order of a number of FISH probes across the relevant region.

The Special Case of the inv dup del(8p)

The inv dup del(8p), noted in the “Biology” section earlier, arises from a maternal cryptic (on classical cytogenetics) paracentric inversion. Yet for a couple who have had this

happen, the risk of recurrence is still, in all likelihood, extremely small. Nevertheless, it would be understandable for a couple having had that experience to seek the reassurance of prenatal diagnosis in a subsequent pregnancy.

The Paracentric Inversion Detected Prenatally.

If an apparently balanced paracentric inversion is discovered at prenatal diagnosis, and if the parental karyotypes are normal, there yet remains a possibility that the rearrangement is not truly balanced, and a risk for abnormality exists. This question is dealt with in detail in Chapter 27 (p. 471).

Notes:

[1] Duplication for a considerably longer segment, 4q31.3→qter, comprising 1.15% of HAL, is viable, as the children in the frontispiece photograph illustrate.

[2] The mother's normal homolog had a short stalk, the inversion a notably long stalk, and the recombinant chromosome in her daughter had a stalk of intermediate length.

[3] These authors raise the intriguing theoretical point that continuing inbreeding in a region with a high prevalence of such a rearrangement could lead to several homozygous individuals being the beginning of a “new” species.







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Chromosome Abnormalities and Genetic Counseling (4 ed.)

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Insertions

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INSERTIONS ARE A TYPE OF TRANSLOCATION: sometimes the expression “insertional translocation” is used. In the common, simple insertion, three breaks are required. The first two breaks release an interstitial segment of chromosome, which is then inserted into the gap created by the third break. In the simple one-way *inter*chromosomal insertion, a segment from one chromosome is intercalated into another chromosome. A more complicated four-break rearrangement is the reciprocal insertion, whereby two nonhomologous chromosomes exchange intercalary segments. In the *intra*chromosomal insertion, a segment is intercalated into another part of the same chromosome. The segment may be inserted “right way around”—that is, with the same orientation to the centromere as before; this is a direct insertion (dir ins). Or it may be reversed—an inverted insertion (inv ins). More complicated scenarios, which may involve both insertional and terminal translocated segments, are more appropriately dealt with in Chapter 12 (Complex Rearrangements).

Insertions are rare rearrangements, at the level of detection according to classical cytogenetics. With microarray technology, previously undetectable insertions of very small size are coming to light, de novo and familial, and it may prove that “uncommon” will be a more accurate adjective to describe frequency than is “rare.” Kang et al. (2010) found a 20-fold increased discovery of insertions, compared with earlier studies, in a large series of cases presenting with typical chromosomal clinical pictures. Many of these turned out to be (probably) harmless polymorphisms (CNVs), upon the recognition of a parent carrying the same insertion. But a fraction were, in all probability, truly pathogenic. It is notable that most of these would not have been detectable on classical cytogenetics.

In this chapter, we consider the case of the phenotypically normal heterozygote, in whom the rearrangement is assumed to be balanced.

The interchromosomal insertion Biology

The simple *one-way* interchromosomal insertion is the most common form of this uncommon rearrangement: Van Hemel and Eussen (2000) estimate a prevalence, on classical cytogenetics, in the order of 1 in 80,000. The formation of the rearrangement is depicted in Figure **10–1**. The recipient chromosome now carries the insertional segment, and the donor chromosome lacks it.

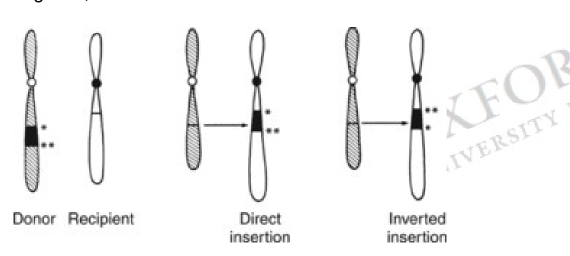


Figure 10–1
The formation of an interchromosomal insertion. Single and double asterisks indicate orientation of the insertion segment. The *direct* insertion has the same orientation to the centromere; the *inverted* insertion has the opposite orientation.

Details of meiotic behavior

In theory, two categories of meiotic behavior are possible, according to whether the homologs pair independently, or as a quadrivalent.

Independent synapsing of homologous pairs

Meiosis could proceed in the usual fashion, with homologs pairing independently as bivalents. In essence, we can suppose that the insertional segment is disregarded and that the homologs synapse, with segments matching for as much of their length as they are able. In theory, and perhaps only with larger insertions, the insertional segment could be thrown into a loop[1] to accommodate this requirement (Fig. **10–2**, upper). (Some crossing-over will presumably occur between synapsed regions, but this would not alter segregation outcomes.) Alternatively, homologs may pair along their full lengths, which would bring some nonmatching segments “incorrectly” alongside each other (“heterosynapsis”).



Figure 10–2
Gamete production following independent pairing of the two sets of homologs. The insertional segment is shown in black, both in its original and in its translocated positions. The horizontal line marks the site whence came the segment from the donor (crosshatched) chromosome, and the site of its destination on the recipient (white) chromosome.

Then, with normal segregation of the two bivalents, independently of each other, two alternative pairs of gametes are possible. Overall, there would be gametes of four possible segregant types, in the ratio 1:1:1:1: two with a correct amount of genetic material, and two without. The former two combinations are 46,N and the balanced insertion carrier.

The two unbalanced combinations would produce conceptuses one with a partial trisomy (duplication) and the other with a partial monosomy (deletion), for the insertional segment (Fig. **10–2**, lower). As discussed later, studies of testicular biopsies and sperm have shown that (at least with smaller insertions) the homologs pair normally as bivalents, and that the expected ratios hold true. It makes no difference whether the insertion is direct or inverted. The foregoing scenario of independent synapsing is more likely to apply when the insertional segment is of small size. The case illustrated in Figure **10–3** exemplifies this: a small (0.4% of haploid autosomal length [HAL]) segment from 8q inserted into 10q, with the duplication and deletion outcomes depicted (this case discussed further later).

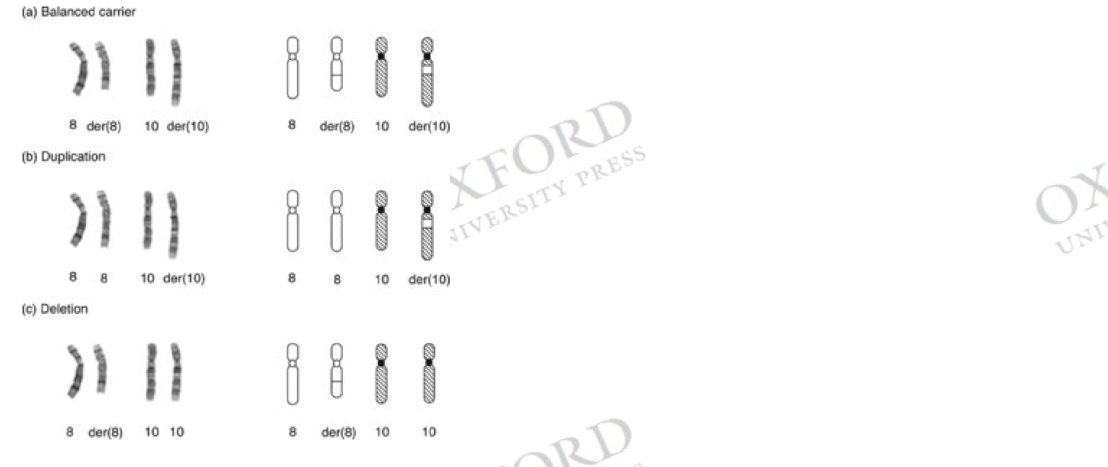


Figure 10–3
An insertion from chromosome 8 to chromosome 10, ins(10:8)(q21;q21.2q22), showing (*a*) the balanced carrier, (*b*) the duplication, and (*c*) the deletion states. In this family, the duplication was the only unbalanced form to be observed. (Case of P. A. Bowen; Bowen et al., 1983.)

Formation of a quadrivalent

Probably only in exceptional cases, with larger insertional segments, a quadrivalent forms, and this would enable recombination within the insertional segments. In the review of Van Hemel and Eussen (2000), the mean size of the inserted segment in recombining cases was 1.5% HAL, compared with 1.0% and 0.5% HAL in nonrecombining families in which the imbalances were due, respectively, to duplication and to deletion. With the *direct* insertion, a recombinant chromosome would be monocentric, and therefore functional. *Inverted* insertions, on the other hand, would be associated with dicentric or acentric recombinant chromosomes, with the resulting gametes predicted to be nonviable.

Consider the large *direct* insertion depicted in Figures **10–4** and **10–5**. Most of the material within the chromosome 5 long arm (q11-q22) has been removed and inserted within the distal long arm of chromosome 1 (Jalbert et al., 1975). A pachytene configuration at meiosis I such as that depicted would allow for complete synapsis of homologous segments. If no crossover occurred in the insertional loop (and assuming 2:2 disjunction with symmetric segregation of centromeres), the same four outcomes noted in the preceding section would eventuate. The gametic combination [a,c] would produce a del(5q11-q22), and the combination [b,d] would produce a duplication for this segment. But if a crossover did occur, two recombinant chromosomes would be formed, and now three further unbalanced outcomes from symmetric 2:2 disjunction would be possible: gametes [b´,d´], [b´,c], and [a,d´] in Figure **10–4**. The duplication/deletion combinations, [b´,c] and [a,d´], are judged to be nonviable, although they might cause miscarriage. The "least imbalanced, least monosomic" combination is the "dup ins" [b´,d´], which leads to a partial trisomy for the insertional segment, 5q11-q22. This was, in fact, the karyotype of the proposita in this family (Fig. **10–5**). Actually, this karyotype endows the same genetic imbalance as would the nonrecombinant [b,d] gamete; so in practical terms, it made no difference that this recombination did happen.



Figure 10–4
Gamete production following formation of a quadrivalent in the interchromosomal insertion, with a single crossover having occurred in the insertion loop. Only one of each sister chromatid is shown. Recombinant chromosomes noted as b´ and d.´ (Based on the case shown in Figure **10–5**.)

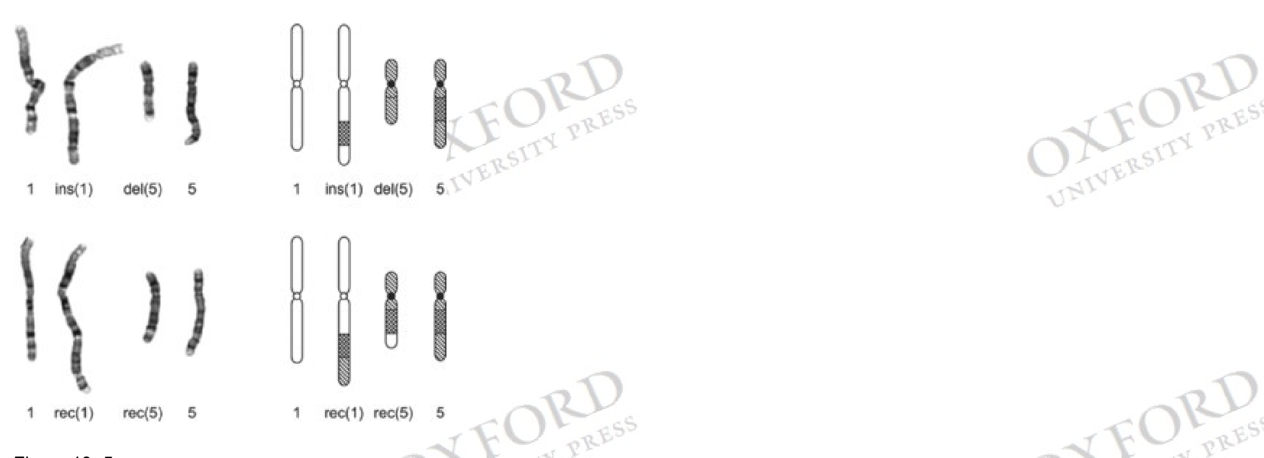


Figure 10–5
Interchromosomal insertion with recombinant chromosomes in phenotypically abnormal offspring. Partial karyotypes of 46, ins(1;5)(q32;q11q22) carrier parent (*above*) and her recombinant child with 46, rec(1)rec(5)dup(5q)ins(1;5)(q32;q11q22) (*below*). The latter is the [b,'d'] combination in Figure **10–4**. The child is trisomic for the segment 5q11-q22. Cartoon karyotype: white, chromosome 1; criss-crosshatched, 5q11-q22; crosshatched, remainder of 5. (Case of P. Jalbert; Jalbert et al., 1975.)

Segment content and viability

The viability of the conceptuses—in other words, the risk to the heterozygote of having an abnormal child—depends on the degree of the aneuploid states. Consider the example illustrated in Figure **10–3**. A small segment from the middle of chromosome 8 long arm (8q21.2-q22) has been removed and is inserted within the chromosome 10 long arm. This segment comprises about 0.4% of HAL. The heterozygote for this rearrangement could produce two types of unbalanced conceptus: one with a duplication of the segment 8q21.2-q22 (Fig. **10–3b**), and one with this segment deleted (Fig. **10–3c**). In this family (Fig. **10–6**), only the duplication was observed. These individuals had mild to moderate mental retardation and minor physical anomalies (Bowen et al., 1983). A segregation analysis of the family was done, and the segregation ratio was close to 1:1:1:0 for normal:balanced:partial trisomy:partial monosomy. This implies a normal viability for the partially trisomic conceptus, and nonviability for the partially monosomic state. Thus, in this family, the risk for having an aneuploid child is 1/1+1+1+0, or 33%. This assessment is an example of a “private” segregation analysis.

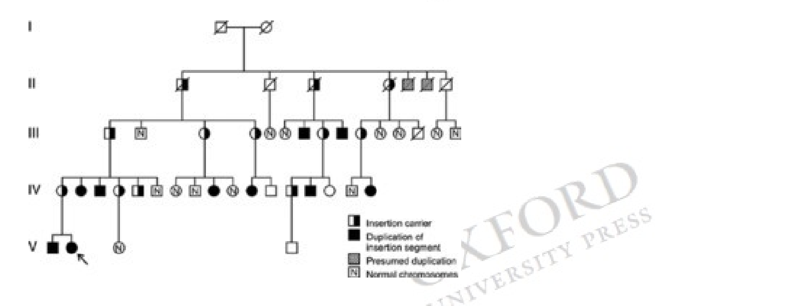


Figure 10–6
The pedigree of the family in which the insertion illustrated in Figure **10–3** was segregating.

A genetically smaller insertional segment has the potential to be viable in both the duplicated and deleted states. For example, Doheny et al. (1997) describe two first cousins, one with a duplication of a segment of 10q, the other with a deletion. The connecting relatives carried an insertion, 46, ins(12;10)(q15;q21.2q22.1).[2] The insertional segment, 10q21.2-q22.1, was small, comprising about 0.5% HAL. The child with the duplication was identified with learning difficulty in first grade, and her IQ measured at 74; the physical phenotype was rather mild. Her cousin with the deletion had, as an infant, considerable lag in neurodevelopmental progress, which would lead one to anticipate a more serious mental defect when the child is older, and she had a more obviously dysmorphic appearance.

An insertion of a very small segment may, on classical cytogenetics, be difficult to detect, although with increasing use of microarray analysis, more such cases will, surely,

come to light (Kang et al., 2010). Löffler et al. (2000) were presented with an adult male thought possibly to have fragile X syndrome. In the event, he had an abnormal chromosome 14, with additional material at band 14q13. His retarded brother and normal mother had the same chromosome. Was this an insertion, an inversion, or what? Fluorescence in situ hybridization (FISH) using microdissection from the abnormal 14 showed a very small hybridizing segment on chromosome 7. Both no. 7 chromosomes in the brothers showed this spot of hybridization, but just one of the mother's. Going back to the G-banded preparations, and now knowing exactly where to look, a deletion at 7q32-q34 could be discerned on the mother's other chromosome 7, and the definitive interpretation could be made. She had the karyotype 46, XX, ins(14;7)(q13;q32q34), and the two sons were 46, XY, der(14)ins(14;7)(q13;q32q34)mat.

In similar vein, consider the insertion in Figure **10–7**, in which two small subbands from 2q (2q33.2 and 33.3) and adjoining parts of q33.1 and q34 are inserted into chromosome 4. This is only about 0.3% of HAL. This rearrangement was at the limit of detection of high-resolution G-banding. In this family, three of five children had a duplication of the insertion, inheriting from the carrier parent the normal chromosome 2 along with the derivative chromosome 4 containing the insertional segment (2q33.1-q34). The children with this very short duplication had a clinical picture of poor speech development, distractable and aggressive behavior, and subtle facial dysmorphism.

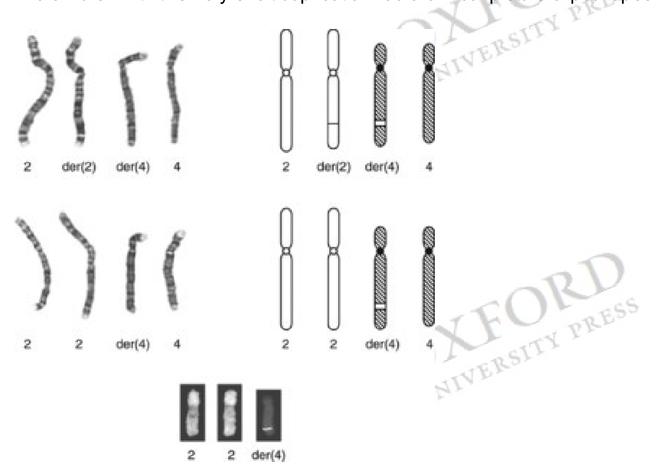




Figure 10–7
A very small insertion, needing fluorescence in situ hybridization (FISH) to be seen clearly. The karyotype of the carrier parent (*upper*) is 46, inv ins(4;2)(q32; q34q33.1). The child is duplicated for the segment 2q33.1-q34, but this is difficult to appreciate on the G-banded karyotype (*middle*). FISH with chromosome 2–specific paint (*lower*) shows the small insertion segment from chromosome 2 present in the der(4). (Case of M. Curtis.)

Although the range of potential abnormalities may be greater in the case of the heterozygote for a large direct insertion because of the additional risks for gametes having recombinant chromosomes, the outlook is not so discouraging in practice. Often the extent of imbalance associated with a recombinant chromosome is so substantial that nonviability is very likely. In other words, these abnormal pregnancies are lost at a very early stage and do not produce an abnormal child. Each family needs to be assessed individually. The counselor may find it useful to follow the format outlined in Figure **10–4** in making this assessment, in terms of what combinations of imbalanced segments might arise, for the large insertion.

The Two-Way Insertion

A two-way reciprocal insertion (a rare observation) has the potential for two different imbalances: a partial monosomy from the segment of one chromosome with the reciprocal partial trisomy of the segment from the other chromosome; or the opposite, with a partial trisomy of one chromosome and a partial monosomy of the other (Fig. **10–8**).

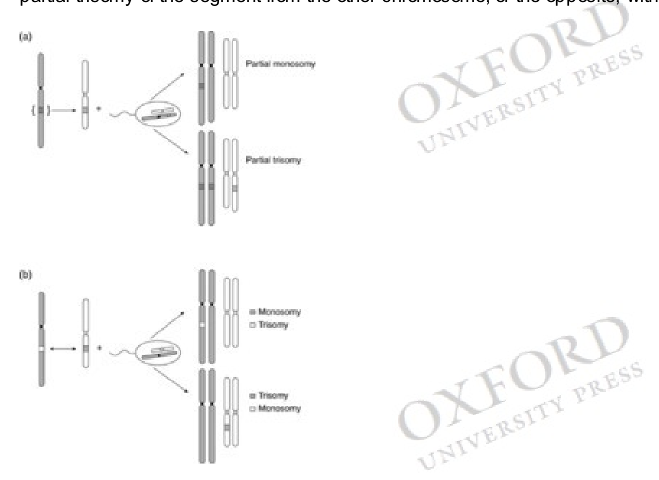




Figure 10–8
Insertional translocations and abnormal segregation. (*a*) One-way, single segment, or nonreciprocal insertion. The hatched bar represents the portion of the chromosome inserted from the gray chromosome to the white chromosome. Upon fertilization by a normal sperm (region of interest from the gray chromosome is shown), there are two possible outcomes (arrows). The upper set of chromosomes shows the outcome after fertilization of an oöcyte carrying a deleted gray chromosome and a normal white chromosome; this leads to a partial monosomy (deletion). The lower set shows the outcome after fertilization of an oöcyte carrying two normal gray chromosomes and a white chromosome with the insertion; this leads to a partial trisomy (duplication). (*b*) Two-way, double-segment, or reciprocal insertion. Upon fertilization by a normal sperm, the upper set of chromosomes shows the outcome after fertilization of an oöcyte carrying only the insertion from the white chromosome. The result is trisomy for the white segment and monosomy for the gray segment. The lower set shows the reciprocal product, with trisomy for the gray segment and monosomy for the white segment.

Instructive Cases

An insertional translocation involving the critical region for Down syndrome provides an interesting illustration that this small segment is indeed sufficient to produce the phenotype. Lee et al. (2005) describe a father with 46, XY, ins(4;21)(q21;q22.13q22.2), who had a child with typical Down syndrome, having inherited the paternal ins(4) with

the small 21q segment, along with two normal chromosomes 21. This case had actually been diagnosed at amniocentesis, when a FISH probe recognizing 21q22 showed three signals; interpretation of the karyotype was a rather more subtle exercise, since 4q21 and 21q22 have similar staining properties, and the small inserted segment thus did not stand out.

Because nonreciprocal insertional translocations lead to single segmental imbalances, they can be helpful in delineating genes or phenotypes. Such is the case of an ins(13;11)(q14.1;p11.2p12) segregating in a family, in which the deletion individuals had biparietal foramina (skull bone deficiencies), multiple exostoses (bone growths), and developmental delay (Shaffer et al., 1993). The description of this family led to the recognition of other deletion individuals, and eventually to the discovery of genes involved in multiple exostoses and biparietal foramina (Wakui et al., 2005; and see Potocki-Shaffer syndrome, p. 318).

Gametogenesis Studies

Gametic analysis has been reported in two insertion heterozygotes. Goldman and Hultén (1992) examined testicular material from an ins(6;7) heterozygote and demonstrated independent synapsis of the chromosome 6 and chromosome 7 homologous pairs at diakinesis, with the two bivalents occupying quite separate parts of the nucleus. This is a direct demonstration that the segregation scenario set out in Figure **10–2** does happen. Testicular tissue and sperm was studied from one ins(3;10) carrier in whom a very small segment of chromosome 10 (p13-p14) was inserted into chromosome 3 at q13.2 (Goldman et al., 1992). In meiosis I, the pairing chromosomes did not loop out the nonhomologous segments; but in fact the normal chromosome 3 appeared to pair fully with the der(3), and likewise the chromosome 10 and the der(10). This may be heterosynapsis. Sperm karyotyping showed, as expected from the theoretical considerations noted earlier, similar proportions of gametes with normal, balanced, duplication, and deletion chromosomes: the actual figures were 22%, 32%, 24%, and 22%, respectively. No recombinant forms were seen. Possibly, small insertions may show similar meiotic behavior, with absence of looping out and no quadrivalent formation. Spermatogenesis may be compromised in some carriers, a conclusion drawn from the observation that only half as many index cases have carrier fathers as they do carrier mothers (Van Hemel and Eussen, 2000).

Rare complexities

Most nucleolar organizing region (NOR) translocations are harmless (see p. [link]). But a NOR insertion into the X chromosome associated with a familial X-linked spastic paraplegia (a condition in which there is stiffness and weakness of the lower limbs, due to neurological deficit at the level of motor neurons in the spinal cord) was apparently pathogenic (Tamagaki et al., 2000). NOR material comprises DNA coding for ribosomal RNA. Two brothers and their maternal uncle had the disease, and the carrier mother was unaffected. Plausibly, the inserted material disrupts a “spinal motor neuron gene” in this region, at Xq11.2, and the male hemizygote, with no gene product being made, thus develops the disorder. It cannot yet be excluded that there is an X-linked mendelian disorder whose locus resides in Xq11.2, cosegregating in the family by chance, and the NOR insertion is simply serving as a cytogenetic marker. The discovery of the gene would prove the point.

There can be a link with cancer if a tumor suppressor gene is located in the insertional segment (Barber et al., 1994). An extraordinary case is seen in a father who had had Wilms tumor as a child, and whose daughter had retinoblastoma, due to an insertion that was apparently balanced in him, and unbalanced in his child. A segment from 13q14 including the retinoblastoma (*RB*) gene was inserted into 11p13, this being the site on chromosome 11 of the *WT1* Wilms’ tumor locus (Punnett et al., 2003).

Genetic Counseling

Insertions are among rearrangements implying the highest reproductive risk. Pooled data from a number of insertion families (Van Hemel and Eussen, 2000) indicate an average risk of having an abnormal child of 32% for the male carrier, and 36% for the female. It may reach 50%. The risk is greater in the small-segment insertion, and smaller in the large-segment. Offering prenatal testing should, in most cases, be the rule. Of the phenotypically normal offspring, approximately half will have normal chromosomes, and half will be insertion heterozygotes. A more detailed discussion follows.

Short insertion segment

For the *short* insertion (say, <1% HAL), the segregation ratio at conception would be expected to be 1:1:1:1 for normal:balanced:duplication:deletion (as discussed earlier). If the insertional segment is not only short but also genetically “small,” both trisomically and monosomically, the maximum risk of having a liveborn aneuploid child would approach 50% (1+1/1+1+1+1). The segment 18q11-q21 (HAL = 0.8%), for example, meets these criteria, as seen in the insertion family presented in Chudley et al. (1974). Carriers for this insertion had all four karyotypic classes of offspring—insertion heterozygotes, karyotypically normal individuals, individuals with a duplication of a small segment of 18q, and individuals with the same segment deleted—in approximately equal numbers. A similar scenario is presented in Marinescu et al. (1999b), with a family segregating an insertion ins(16;5)(q22; p14p15.3). Here, the “small” segment comprised 5p14-p15.3. In two generations from a heterozygous grandparent, there were two children with 5p–, two with 5p+, four normals, and three carriers. The same level of risk, with a 1:1:1:1 segregation as earlier, is also likely to apply to the very small insertion that requires microarray for its recognition.

If viability is reduced or impossible for the trisomic or monosomic conceptuses, the risk would be correspondingly less. Trisomic lethality presumably increases with an increasing fraction of HAL, with monosomic imbalances being more lethal.

It may not be possible to make a clear judgment, based on the literature, about the qualitative content of the imbalance, because the insertion involves an interstitial segment of chromosome, whereas most data on record relate to distal segments. A review of the insertional data on record up to 2000, taken from nearly 90 families, is provided in Van Hemel and Eussen (2000), and Figure **10–9** is taken from their paper. Any insertion involving the same open bar (deletion) or filled bar (duplication) segment, or part thereof, will have a significant risk. Schinzel's (2001) cytogenetic database and the Internet sources ECARUCA and UKCAD (p. 309) may also be consulted; some more distal insertional segments may well be bounded within terminal duplications and deletions that have been described in liveborn individuals. Of course, any unbalanced child in the counselee's family will provide proof of viability, and an illustration of that particular phenotype. A study of the wider family may provide a guide to the recurrence risk—a “private” segregation analysis, as illustrated earlier in the “Biology” section. But in any case, the starting point with a patient having a short insertion is that the risk for an abnormal child is high, by which we mean in the range 10%–50%.



Figure 10–9

(*Above and opposite*) Presentation of chromosome segments in which recombinant imbalances have been recorded (on classical cytogenetics), in the child of a parent heterozygous for an interchromosomal insertion. Segments seen only as duplications are shown in filled bars, those seen only as deletions in open bars, and filled and open bars connected show segments observed in either state. Insertions seen only in the balanced state are identified with striped bars. (From Van Hemel and Eussen, 2000, Interchromosomal insertions. Identification of five cases and a review, *Human Genetics* 107:415–432. Courtesy J. O. Van Hemel; with the permission of Springer-Verlag.)

Longer insertion segment

For the direct insertion involving a *longer* segment (say, >1.5% HAL), there is theoretically an additional risk for the formation of recombinant duplication and deletion chromosomes. But in fact the deletion for a long segment (whether the result of a nonrecombinant or recombinant chromosome) would usually impose a nonviable degree of partial monosomy. The dup/del combinations (see Fig. **10–4**) are even more unbalanced, leading to spontaneous abortion. Thus, only the duplication (whether nonrecombinant or recombinant) is likely to allow for viability. In the great majority of cases, therefore, the segregation ratio for pregnancies going to term is 1:1:x:0 for normal:balanced:partial trisomy:other imbalances, where x is less than 1, and probably very much less than 1.

In the family of Jalbert et al. (1975) discussed earlier (Fig. **10–5**), the insertional segment (5q11-q22) comprised 2.2% HAL, and this duplication did allow survival, although the child was dysmorphic and severely mentally retarded. This case is the sole example of dup(5)(q11-q22) in Schinzel's database; this specific segment is not listed in ECARUCA nor in UKCAD. The risk for recurrence in this family, or occurrence in another family, must surely be small, and perhaps x is only a low single-digit number. In a family such as that in Abuelo et al. (1988), with an insertional segment comprising most of 3p (p26-p13, 2.5% HAL), one could be rather confident that any imbalanced conception would miscarry. The closest viable segment in Schinzel's database is 3pter-p14, and there are only two cases of this listed; in ECARUCA, the closest match is 3p26-p21.3, a single case. A risk of “almost 0%” for an abnormal child could be offered. Prenatal diagnosis in cases judged to be of this very low risk category would be discretionary; a normal ultrasonographic fetal anatomy scan would likely be considerably reassuring in itself.

Intermediate length segment

Intermediate length segments (1%–1.5% HAL) might imply a risk in the range 5%–10%. But each segment needs to be judged on its merits, both according to the reproductive history in the family, and with reference to the cytogenetic databases.

Preimplantation Genetic Diagnosis

Preimplantation genetic diagnosis in the context of an insertion presents challenges. If the theory of quadrivalent formation, with possible crossing-over, is correct, the use of additional probes would be necessary to account for this (Melotte et al., 2004).

The Intrachromosomal Insertion **Biology**

Intrachromosomal insertions, also known as “centromere shifts,” are very rare, with only about 40 cases published (Madan and Menko, 1992; Ardalan et al., 2005). The cytogenetic recognition can be difficult, with some having originally been interpreted as paracentric inversions with unbalanced meiotic products (Madan and Nieuwint, 2002). The formation of the intrachromosomal insertion is outlined in Figure **10–10**. These insertions can be within-arm or between-arm, and direct or inverted, and they may undergo incomplete or complete synapsis. These differences can have practical consequences, and we need to consider each in turn.

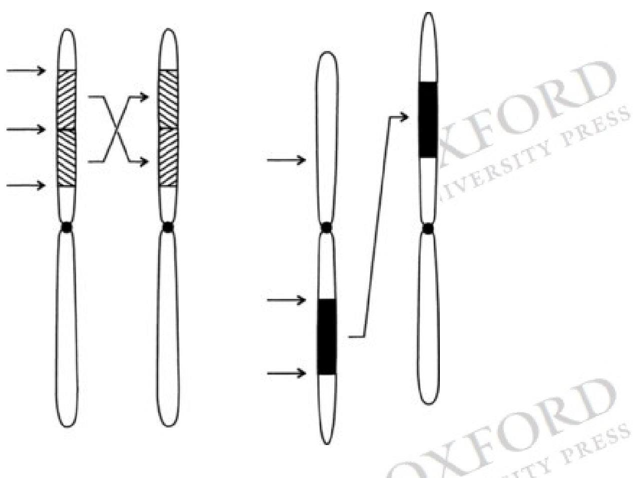




Figure 10–10
The formation of the intrachromosomal insertion. *Left*, the within-arm insertion, with the inserted segments crosshatched. The normal chromosome is on the left, and the insertion chromosome on the right. *Right*, the between-arm insertion, with the inserted segment in black. The normal chromosome is on the left, and the insertion chromosome on the right. Compare with the ins(5) shown in Figure **10–15** and the ins(5) in Figure **10–16**, respectively.

Within-Arm Insertion

A shift of chromatin within the same arm is called, logically enough, a within-arm[3] insertion. Since both segments shift, essentially switching positions, each could be called an "inserted segment." If both segments maintain the same orientation toward the centromere, it is a *direct* insertion. If the orientation of one segment is reversed, it is an *inverted* insertion. In the case of the inverted inversion, we can distinguish one segment from the other by referring to respective inverted and noninverted segments. In the direct insertion, the shorter of the two segmentscan be arbitrarily labeled as the inserted segment, and the longer as the "noninserted" or "interstitial" segment (Madan and Menko, 1992; Barber et al., 1994); since they are both really insertion segments, we can also speak of the "shorter inserted" and the "longer inserted" segments. Array comparative genome hybridization (CGH) enables, in the unbalanced case, a subtler discernment of the inserted segment (Burnside et al., 2009).

Between-Arm Insertion

The other type is the between-arm[4] insertion, with a segment of chromatin from one arm inserted into a point in the other arm (Fig. **10–11**). If we consider the part of the chromosome containing the centromere as the fixed reference point of a chromosome, we can regard the centromeric segment as "staying still," while the insertion segment shifts from one arm to the other. This somewhat arbitrary point of view allows us to use the term "inserted segment" unambiguously, in the context of the between-arm insertion. Thus, in Figure **10–11**, the segment shown in black has moved "up" from the long arm and is inserted into the short arm (rather than the segment containing the centromere moving "down" into the long arm).[5]

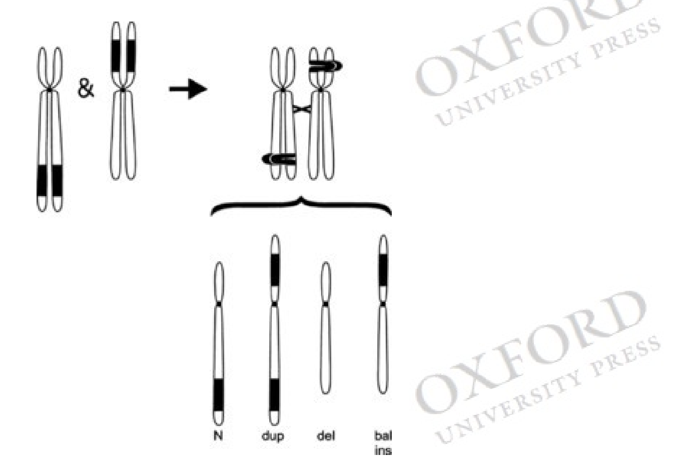




Figure 10–11
Gamete production following a recombination *between* the sites of rearrangement in the between-arm intrachromosomal insertion. At the top of the figure, the normal chromosome is on the left, and the insertion chromosome on the right. There is incomplete synapsis, with ballooning out. (Based on the ins(5) shown in Figure **10–16**.)

Incomplete synapsis

Meiosis perforce proceeds in a modified fashion. Consider the *between-arm* shift. In most cases, perhaps, the inserted segments fold out so as to allow a good degree of synapsis of the bivalent. This synapsis would include that part of the chromosome between the two inserted segments, that is to say, the centromeric segment. There would be no difference, at least in theory, if the insertion is direct or inverted. One (or any odd number) crossover within the centromeric segment will produce recombinant chromosomes: one with a duplication of the insertion segment, and the other with a deletion (Fig. **10–11**). The centromeric segment may be quite long, as a proportion of the whole chromosome, and provide considerable opportunity for crossover. Thus, the genetic risk is expected to be high; and in theory could approach 50%. In other words, the segregation ratio for the four possible segregant outcomes of normal:balanced insertion:duplication: deletion would be close to 1:1:1:1. According to the level of in utero genetic compromise imposed by the deleted and duplicated states, respectively, the risk for an abnormal outcome in a liveborn child may be correspondingly less. The family illustrated in Figure **10–12** has an intrachromosomal insertion involving the "Potocki-Shaffer segment" (11p11.2), small enough that microarray is needed for its demonstration. With likely no reduced viability of either imbalanced state in utero, the risk to the carrier here may indeed be 50%.



Figure 10–12
Family tree (*a*) showing segregation of an intrachromosomal insertion ins(11)(q23.1p11.2p12), with both deletion and duplication observed in the family, and (*b*) cartoon karyotype to show the nature of the rearrangement. The insertion was detected on BAC microarray-based CGH, and the insertional segment is of approximately 2 Mb in length. Half-filled symbol, balanced carrier; filled symbol, 11p deletion (Potocki-Shaffer syndrome); crosshatched symbol, 11p duplication. The formal karyotypes of the deletion and duplication states are rec(11)del *or* dup(11)(p11.2p12)ins(11)(q23.1p11.2p12). (Case of J. Gastier-Foster and C. Astbury.)

The *within-arm* shift, in the case of the direct insertion, can have a similar folding out of one inserted segment, and its homolog on the normal chromosome, to enable synapsis of the other inserted segment and its homologous region. In Figure **10–13**, we depict the shorter insertion segment folded out, with synapsis of the larger inserted segment (it could have been drawn the other way around). Recombination within the larger segment will lead, respectively, to duplication and deletion of the shorter segment in the recombinant products passed on to the two resulting gametocytes. Vice versa, if there is synapsis of the shorter inserted segments, followed by recombination, there would be duplication of the larger inserted segment in one gametocyte, and deletion of this segment in the other. In theory, the longer the larger segment is, the more likely it is that recombination will happen; but nevertheless, cases are on record of crossing-over taking place in very short inserted segments, in both direct and inverted insertions (Webb et al., 1988; Rethoré et al., 1989; Barber et al., 1994). Theoretically, if this process were to happen with an inverted insertion, dicentric and acentric products, almost certainly nonviable, would result.

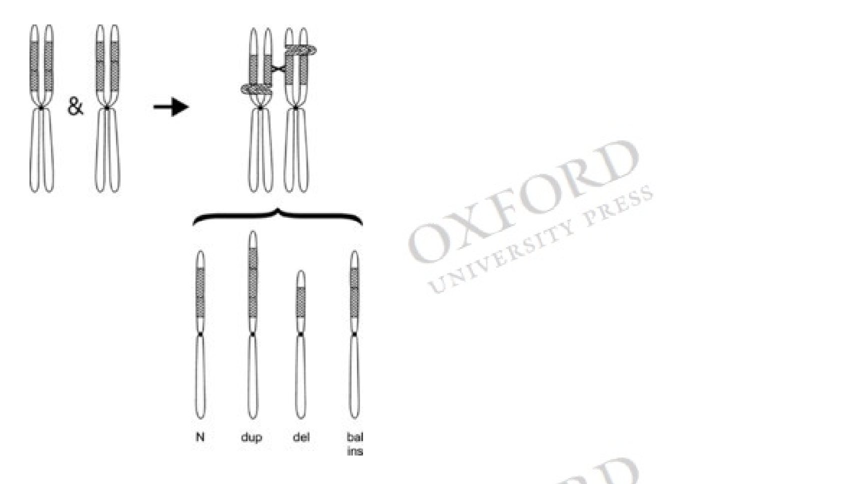


Figure 10–13
Gamete production following a recombination *within* one of the insertion segments (the longer segments) of a direct within-arm intrachromosomal insertion. There is incomplete synapsis. There are four possible gametic outcomes. Compare with the ins(5) shown in Figure **10–15**, although note the subtle difference that in the latter the recombination took place between the shorter inserted segments.

Complete synapsis, direct insertion

Alternatively, and likely only in the case of the inserted segment being of large size, complete synapsis may be achieved. The insertion and the centromeric segments (between-arm shift) or the two insertion segments (within-arm shift), and their matching segments on the normal homolog, would need to loop back and forth into each other, forming a double loop (Fig. **10–14**). Various outcomes are possible from crossing-overs within one or other loop. Considering the *direct between-arm* shift, crossing-over within the *centromeric* segment will lead to recombinant chromosomes deficient or duplicated for the inserted segment (Fig. **10–14a, b**). If, however, following complete synapsis, there is crossing-over within the *inserted* segment, this will lead to the generation of new recombinant forms: chromosomes that are duplicated for terminal p and deleted for terminal q, or vice versa (Fig. **10–14c, d**). A notable such example is illustrated in Ardalan et al. (2005), concerning a mother who carried a dir ins(20)(p13q11.21q13.33) (initially thought to be a pericentric inversion). The “shifted” segment was relatively large, about half the length of the chromosome, and the del qter/dup pter recombinant karyotype conveyed a survivable imbalance.



Figure 10–14
The range of possible recombinants from crossing-over in one or other insertion loop following complete synapsis of the intrachromosomal insertion. The four panels show, from above down, the direct between-arm insertion, the inverted between-arm insertion, the direct within-arm insertion, and the inverted within-arm insertion. In the loop diagrams, the dots signify the centromere, and the × shows the point of crossover. The insertion segment DE is shown in thick line in the loop and in the recombinant chromosomes. Circled letters provide reference points for text comments. (Adapted from Madan and Menko, 1992.)

If complete synapsis is achieved in the *direct within-arm* shift, there is no new category of recombinant form beyond the four that could be generated from incomplete synapsis with folding out of one of the segments. Crossing-over within the longer inserted segment will lead to recombinant chromosomes deficient or duplicated for the shorter inserted segment (Fig. **10–14i, j**). Vice versa, crossing-over within the shorter inserted segment will lead to recombinant chromosomes deficient or duplicated for the longer inserted segment (Fig. **10–14k, l**). We illustrate such a case from Webb et al. (1988) in Figure **10–15**; equally, this outcome could have arisen from incomplete synapsis, with the longer segments folded out.

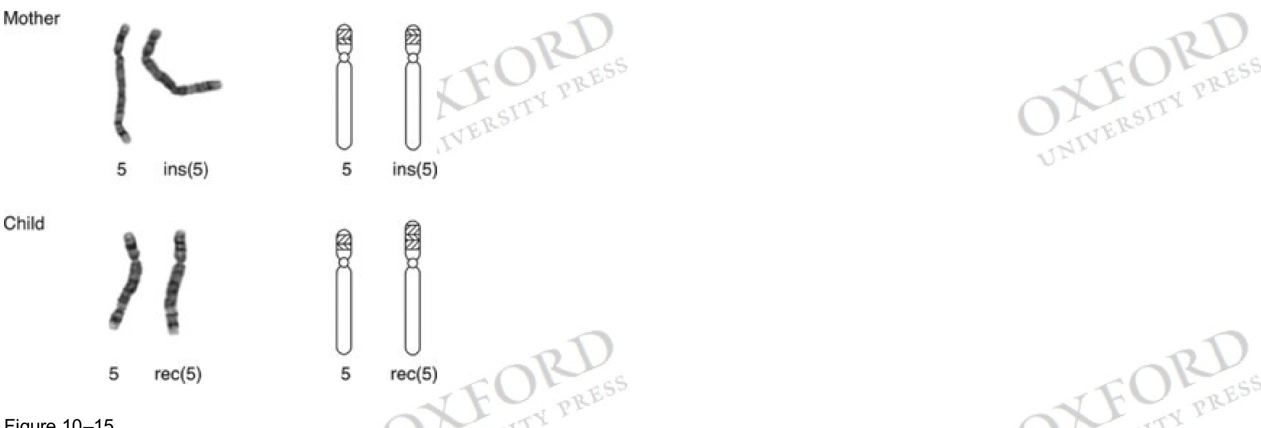


Figure 10–15
Recombination from a direct within-arm shift. Partial karyotypes of an insertion heterozygote mother, and her recombinant child. The karyotypes are 46, dir ins(5)(p14.1p14.3p15.1), and 46, rec(5)dup(5p)dir ins(5)(p14.1p14.3p15.1)mat.[6] The child is duplicated for 5p14.3-p15.1, shown as the larger crosshatched segment. The recombination may have arisen from crossing-over within band p14.1 (smaller crosshatched segment) at either partial synapsis with ballooning out of segments p14.3-p15.1, as in Figure **10–13**, or from complete synapsis following double-loop formation, as in Figure **10–14*k***. (Case of L. E. Voullaire; Webb et al., 1988.)

Complete synapsis, inverted insertion

Recombination in the inverted *between-arm* insertion, in the setting of complete synapsis, has the same consequences as for the direct insertion as discussed earlier, when crossovers take place within the *centromeric* segment (Fig. **10–14e, f**). The family illustrated in Figure **10–16** demonstrates this. The recombinant child with a dup(5) could equally have arisen from recombination in a partial synapsis (Fig. **10–11**) or in a complete synapsis (Fig. **10–14f**), but in either event the crossover is within the centromeric segment. The duplication comprises the inverted insertion segment. If, however, the crossover is in the *inserted* segment, dicentric and acentric products will result, and, if a zygote were to result from such a gamete, the compromised conceptus will probably degenerate very early and may not even implant (Fig. **10–14g, h**). The same fate awaits conceptions from crossovers in the inverted *within-arm* shift, if crossing-over happens within the *inverted* segment (Fig. **10–14o, p**). If crossing-over is in the *noninverted* segment, we see the same imbalances (Fig. **10–14m, n**) as in the direct within-arm shift (Fig. **10–14i, j**). Thus, Rethoré et al. (1989) describe a child with a duplication for the very short segment 5p13.32-p14.2 due to a parental inv ins(5)(p13.31p14.3p15.12) with recombination in the even shorter segment p14.3-p15.11, reflecting the scenario set out in either Figure **10–14n**, or in Figure **10–13**.

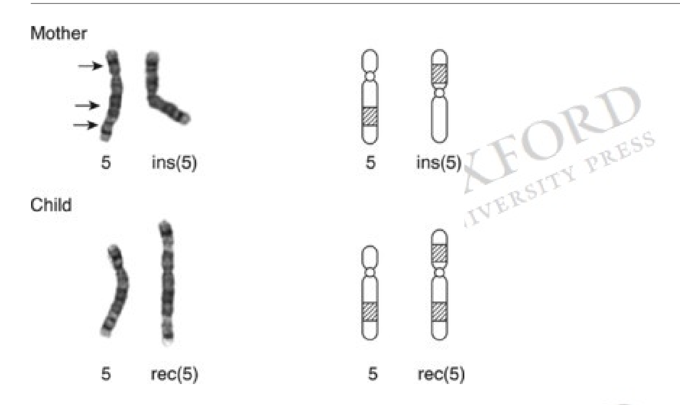




Figure 10–16
Recombination from an inverted between-arm shift. Partial karyotypes of an insertion heterozygote mother and her recombinant child. The karyotypes are 46, inv ins(5)(p13q22q33), and 46, rec(5)dup(5q)inv ins(5)(p13q22q33)mat. The child is duplicated for 5q22-q33 (indicated by the crosshatched segment). The recombination may have arisen from crossing-over anywhere between 5p13 and 5q22 at either partial synapsis with ballooning out of segments 5q22-q33, as in Figure **10–13**, or from complete synapsis following double loop formation, as in Figure **10–14f**. (Case of N. J. Martin; Martin et al., 1985.)

A notable example of a three-generational inverted within-arm insertion is the inv ins(15)(q15q13q11.2) family described in Collinson et al. (2004). The grandmother, her son and her daughter, and one grandchild were heterozygous for the insertion, with the detailed karyotype written inv ins(15)(pter→q11.2::q13→q15::q13→q11.2::q15→qter). Three grandchildren were abnormal: one with Prader-Willi syndrome (PWS), one with the dup(15)(q11-q13) syndrome (p. 329), and the third with Angelman syndrome (AS). As the reader may already have guessed, the AS grandchild was born to the carrier daughter, while the PWS grandchild was fathered by her son: these two grandchildren had each inherited a deletional rearrangement. The grandchild with the dup(15)(q11-q13) syndrome, 46,XX,rec(15)dup(15)(q13-q11.2)ins(15)(q15q13q11.2)mat, was the carrier daughter's child, having inherited a duplicational rearrangement; the maternal origin of the duplication was the reason, presumably, for that grandchild's abnormality. The rearrangements would have arisen following either the scenario set out in Figure **10–13** or Figure **10–14***m* (the deletion) or 10–14*n* (the duplication).

The reader may have discerned a pattern in the aforementioned construction. Whichever segment recombination takes place in (the active segment, so to say), it is the *other* (passive) segment that comes to be duplicated or deleted. This is logical. A crossover will create a new version of the active segment that contains a portion from each contributing chromosome—but it will be the same length as it was before. The other, non-crossing-over segments follow, as it were, passively along.

Complete synapsis, insertion segments in both directions

If the insertional segment is itself rearranged, such that one part is in direct orientation, and the other inverted, this is an "intrasegmental double inversion," and represents a four-break, rather than a three-break rearrangement (Wang et al., 2010a); in which case, Figure **10–14c** would need to redrawn, following Figure 2 in Wang et al. This reinterpreted crossing-over configuration might allow a closer coming-together of the two segments in the normal and the insertional homologs, and possibly be the basis of a particularly high risk of producing imbalanced gametes.

Concerning the X chromosome, if the "critical region" of the X is involved, an insertion may, in spite of being balanced, produce gonadal dysfunction in the female (Grass et al., 1981).

Genetic Counseling

The risk to have an abnormal recombinant child, in the 27 families reviewed by Madan and Menko (1992), was 15%, although they considered this quite possibly to be an underestimate. This is an average figure, and it was derived from families studied with classical cytogenetics. We may presume a range from near 50% to zero in the individual case. A high risk is likely if one of the segments is small, and the other long, so that *(1)* there is a high survivability in both the duplicated and deleted state for the small segment, and *(2)* with one long segment, recombination may be more likely to take place. In this situation, a figure of 30%–40% may be the appropriate one to offer. Given that the partial aneuploid states will involve interstitial regions of the chromosome, very little data, quite possibly none, may be on record for the viability and phenotype of the particular segment (but of course the appropriate databases should be checked); and an educated assessment will have to be made. In the case of very small insertions, detectable at the level of microarray, the risk is likely to be at the upper end of the range.

Risks are presumably less, and possibly zero, if both segments are long (that is, no recombinants are viable). The risks *may* also be less—say, below 10%—if both segments are short, which might weigh against recombination; but we have no firm data to buttress this suggestion. As always, a "private" segregation analysis, if the family offers that opportunity, may provide the best estimate of risk. For one specific insertion, Allderdice et al. (1983) calculated a risk of 31% for female inv ins(9)(q22q34.3q34.1) heterozygotes. But prediction is imprecise. One short-segment between-arm shift, 46, dir ins(7)(p22.1p21.4q36.1), with a long centromeric segment for which, from the foregoing, a high risk might have been predicted, in fact produced no liveborn recombinant child in a three-generation family, although some first- and second-trimester pregnancy losses may have been due to unbalanced forms (Farrell and Chow, 1992).

Notes:

[1] Described also as ballooning out, looping out, folding out, or translocation loops.

[2] In the ISCN nomenclature, the recipient chromosome is noted first, followed by the donor chromosome.

[3] Also called intra-arm, and paracentric insertion.

[4] Also called inter-arm, and pericentric insertion.

[5] It may be more complex than this: Wang et al. (2010a) propose that an inserted segment might invert within itself, as a four-break, rather than a three-break rearrangement.

[6] This karyotype stretches the limits of the short nomenclature, since "dup p" could refer to either 5p14.1 or 5p14.3–15.1. The full nomenclature describes the rearrangement: 46, XX,–5,+rec(5)(pter-p14.1::p15.1-p14.3::p13.3-qter),dir ins(5)(p14.1p14.3p15.1)mat.







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Chromosome Abnormalities and Genetic Counseling (4 ed.)

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Autosomal ring chromosomes

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RING CHROMOSOMES ARE UNCOMMON, and it is even more uncommon for a person with a ring (or someone on their behalf) to seek genetic advice about reproductive possibilities. The typical physical phenotype comprises major dysmorphogenesis and mental retardation, and procreation is not usually a relevant issue. But exceptions exist. Remarkably, some persons with a ring chromosome seem to be of entirely normal phenotype. Only mild mental retardation, or short stature with minor dysmorphism, characterizes some other cases. The ring 20 has a unique association with epilepsy. It is these categories of normal or mildly abnormal phenotype—in other words, of possible reproductive potential—we particularly consider in this chapter, although at the outset we can state that only a few examples of parental transmission of ring chromosomes are known. About 99% of rings arise sporadically (Kosztolányi et al., 1991). The ring X Turner syndrome variant is noted in Chapter 13, and the "tiny ring X syndrome" on p. 484.

Biology

There are two major types of ring chromosome that can be associated either with a normal phenotype, or with a clinical picture of relatively mild mental compromise, growth restriction, and absence of major malformation. First, the full-length or nearly full-length ring that replaces one of the normal homologs with the karyotype 46,(r). Second, the very small ring comprising pericentromeric chromatin, which exists as a supernumerary chromosome, with the karyotype 47,+(r). Individuals with either of these types of ring may have intact fertility and may present with questions about risks to their offspring. A third type of ring, in which the phenotype would always be abnormal, may have a complex structure at the breakpoint junctions with terminal deletions and duplications (Rossi et al., 2008b); we do not further discuss this category. We deal with the first two categories separately and list some reported cases of individual ring chromosomes.

The Apparently Balanced Ring Chromosome, 46,(r)

We may list these theoretical mechanisms that could lead to the generation of a ring that might appear, on classical cytogenetics, to be balanced:

(1) Fusion of telomeres, without loss of other chromosomal material
(2) Deletion of subtelomeric material at terminal p and/or q arms, with fusion of the exposed ends, with only the repetitive telomeric segments lost (telomere healing)
(3) Deletion of "small" amounts of euchromatin at p and/or q arm, with fusion of the exposed ends

In the first two scenarios, no genes are lost, and no haplo-insufficiency is imposed. In the third case, and if the deletions included actual genes, and if these genes were dosage sensitive, then there would be a phenotypic effect. Further, the circular structure of the ring of itself would then compromise postzygotic mitotic cell division. The rings would become entangled, broken, doubled, or otherwise disrupted, following sister chromatid exchange during the cell cycle (Fig. **11–1**). Thus, daughter cells arise that would be partially or totally aneuploid (whether trisomic or monosomic) for the chromosome in question—"dynamic mosaicism." These cells might die; some, however, could survive in the mosaic state and presumably make an unfavorable contribution to the phenotype. This continuous generation and loss of cells would seriously undermine the growth *rate*, although it might not greatly influence the quality of growth. The result would be the "general ring syndrome"—whichever autosome is concerned—of marked growth retardation, mild to moderate cognitive impairment, minor dysmorphogenesis, and, perhaps, intact fertility; intriguingly, café-au-lait macules are quite often seen (Kosztolányi, 1987; Sodré et al., 2010). An alternative view is that genomic imbalance suffices, of itself, to lead to phenotypic abnormality, in some cases, at least; and that there is no need to invoke a general ring syndrome (Rossi et al., 2008a). Zollino et al. (2010b), with specific reference to the r(14), propose that silencing of genes may be contributory.

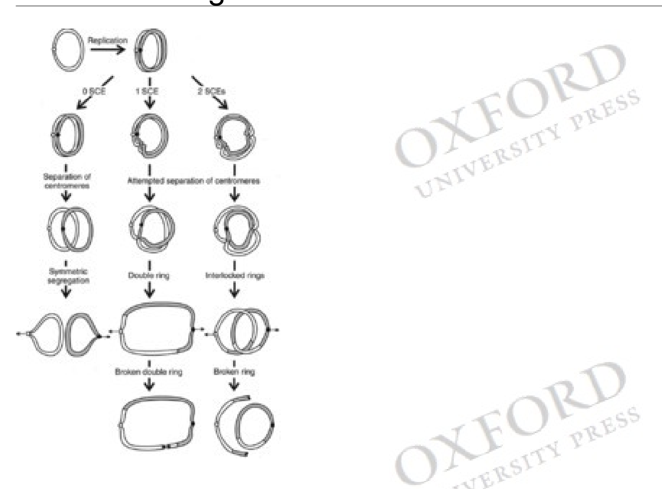




Figure 11–1
Dynamic mosaicism. The single-chromatid ring chromosome replicates during interphase. Sister chromatid exchanges (SCEs) may, or may not, take place. At meiosis, if there are no SCEs (*left*), segregation is symmetric (dotted arrows represent spindles drawing homologs to opposite poles). If there is one SCE, a double-sized ring is generated (*middle*). With each centromere being tugged to opposite poles at anaphase (dotted arrows), the chromosome may break. If there are two SCEs, in the same "direction of rotation" (*right*), the two rings become interlocked. Breakage, or other mechanical compromise, is the consequence. A second SCE in the opposite direction of rotation would restore the situation.

Details of Meiotic Behavior

At gametogenesis in the 46,(r) heterozygote the expectation is, other things being equal, for symmetric disjunction, with 1:1 segregation of the ring and the normal homolog (Fig. **11–2**). Thus, half of the conceptuses would be entirely normal karyotypically, and half would carry the ring. If "dynamic mosaicism" then occurred, these latter may be lethal in utero, or those surviving to term might have phenotypic abnormality.

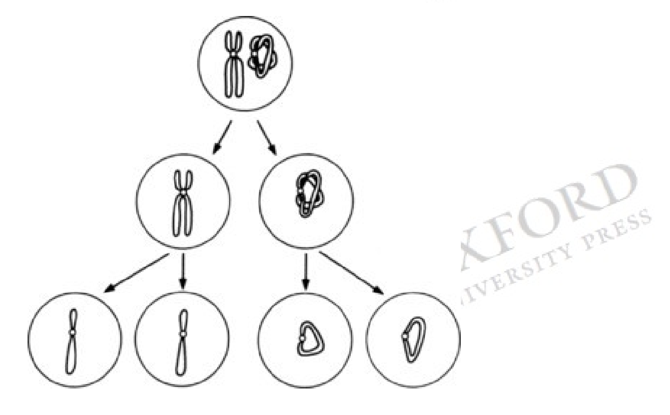




Figure 11–2
Meiosis with symmetric segregation in the ring heterozygote.

There are tentative grounds for considering that the ring heterozygote might have an increased risk for nondisjunction, resulting in 2:0 segregation. In this event, with respect to chromosomes 13, 18, or 21, a child with the respective trisomy might be born. So far, this is on record only in the case of a child with ring Down syndrome, 47,+r(21), born to a 46,r(21) parent (Kosztolányi et al., 1991). The r(21) parent is also at risk of having a Down syndrome child due to a recombinant duplication 21 (Howell et al., 1984; Fryns and Kleczkowska, 1987; Miller et al., 1987).

Almost all instances of parent-to-child ring transmission involve the mother as the carrier parent (MacDermot et al., 1990). Probably, spermatogenesis is compromised in the presence of a ring chromosome, and infertility is the consequence for most male heterozygotes.

Most of the autosomes are represented in the list of rings, in the form 46,r(A). In a few there has been an association with phenotypic normality and parenthood, and we provide commentaries later. (As noted earlier, there is sometimes mosaicism, more usually with one cell line monosomic for the chromosome concerned, and occasionally a minor cell line with two copies of the ring.)

Ring 1, 46,r(1).

Few reports exist (Gardner et al., 1984; Cutenese, 2000). Growth retardation is typical.

Ring 2, 46,r(2).

Prenatal and postnatal growth retardation and microcephaly are consistent features. We have seen a case of 46,(r)(2)/46,N mosaicism, a profoundly retarded girl, with dramatic levels of dynamic mosaicism: 22% of cells were tetraploid, and most of the derivative rings were in the tetraploid cells (Sutherland and Carter, 1978). Lacassie et al. (1999) summarize eight published cases and provide a photographic record of their own patient from birth to age 10 years, a microcephalic child with some mild cognitive and behavioral compromise, and profoundly growth retarded. Dee et al. (2001) showed a subtle distal 2p deletion in a ring 2 child with a similar phenotype, and they suggest that some other cases of r(2) may also have very small deletions. It thus remains an open question whether the phenotype is truly a manifestation of the general ring syndrome, or that it is due, in part at least, to a distal deletion. No parent to child transmission has been recorded.

Ring 4, 46,r(4).

Sigurdardottir et al. (1999) describe a growth-retarded infant with normal developmental progress and whorled areas of hyperpigmentation and hypopigmentation. The r(4) was a

true telomere-to-telomere fusion, as demonstrated with fluorescence in situ hybridization (FISH) using subtelomeric probes. If the ring formed following terminal deletions, the individual might or might not present a Wolf-Hirschhorn syndrome phenotype, according to the extent of the deletion into 4p (Balcı et al., 2006; Kim et al., 2009). We have seen a man with 46,XY,r(4) manifesting the "general ring syndrome": he was considerably shorter than his brothers, and his occupation of warehouse manager compared with the professional qualifications of his siblings. Nevertheless, he could fully appreciate the genetic implications of his condition, and he and his wife chose to have donor insemination.

Ring 6, 46,r(6).

Urban et al. (2002) reviewed 23 cases. Hydrocephalus was a common observation. At one end of the spectrum, malformations and microcephaly with severe retardation are typical. Kara et al. (2008) describe epilepsy as part of the phenotype in a patient whose r(6) included a 6q deletion. At the other end, a much milder phenotype of growth retardation evokes the general ring syndrome. An example is provided by the case of a young woman with mild dysmorphism and short stature, but normal psychomotor development and intact fertility (her son had a normal karyotype), reported in Höckner et al. (2008). The r(6) had approximately 200 kb deleted from each arm of the chromosome. No instance is known of parent-to-child transmission of a r(6).

Ring 7, 46,r(7).

In a review of 16 cases of r(7), most had presented with microcephaly and intellectual deficit (Kaur et al., 2008). Vermeesch et al. (2002) described a patient with microcephaly and height well below the third centile; a few cells had double rings. In this case, they demonstrated a subtelomeric fusion: FISH probes for subtelomeric sequences gave positive staining on the ring chromosome, but no staining occurred with telomere probes.

Ring 8, 46,r(8).

A man having a ring 8 with megabase-size deletions at 8pter and 8qter, and whose intellectual deficit was less marked than in most r(8) cases, proved to be mosaic, with a upd(8)pat 46,XY cell line (Gradek et al., 2006). The probable sequence was as follows: 46,r(8) at conception, the ring of maternal origin; mitotic loss of the ring to give a 45,–8 cell; and subsequent "rescue" of the monosomic line by duplication of the normal (paternal) homolog. This mechanism may have operated in some other ring cases in which there is a concomitant normal cell line.

Ring 9, 46,r(9).

The phenotype in the r(9) is comparable to that of deletion 9pter and 9qter cases (Purandare et al., 2005; Sheth et al., 2007). Common elements include dysmorphism, microcephaly, cardiac malformations, growth and psychomotor retardation, and skeletal anomalies. A particular feature may be ambiguous genitalia or, sometimes, sex reversal, caused by deletion of *DMRT1* on 9p. The karyotype has been reported in a case at prenatal diagnosis, in which subtelomeric 9p and 9q deletions were proven; fetal defects were documented post termination (Chen et al., 2006d).

Ring 10, 46,r(10).

Gunnarsson et al. (2009) report a girl with growth and psychomotor retardation, microcephaly, congenital heart defects, and dysmorphic features. The nonmosaic ring was characterized by array comparative genome hybridization (CGH) and showed terminal deletions of 12.5 Mb at 10q26.12-qter, and 285 kb at 10p15.3-pter.

Ring 11, 46,r(11).

A mildly dysmorphic and developmentally delayed child had a ring 11 in which the 11p15.5–11p15.4 segment was duplicated, and although this was of paternal origin, he did not present a Beckwith-Wiedemann phenotype (p. 360), but he did develop bilateral Wilms tumor (Carella et al., 2010).

Ring 12, 46,r(12).

Parmar et al. (2003) review the findings in six cases of 46,r(12). Growth retardation and intellectual compromise of varying degree were consistent features. In one 46,XY,r(12)(p13q24.3)[85%]/46,XY[15%] mosaic case, a man in his twenties presented with infertility associated with severe oligospermia; the diagnosis led to retrospective review, and it was noted that he had been assessed as a child for delayed learning and microcephaly (Martin et al., 2008). He also had a number of café-au-lait skin macules, misleadingly the basis of a previous diagnosis of neurofibromatosis; but as noted earlier, this sign is observed in a number of ring chromosome syndromes.

Ring 13, 46,r(13).

The typical phenotype, due to the distal 13q deletion component of the ring, presents microcephaly and poor psychomotor development, and genital malformation (Walczak-Sztulpa et al., 2008). Bedoyan et al. (2004) report mother-to-daughter transmission of a ring 13 chromosome, in which there had been loss only of subtelomeric material. The mother attended a special school; at age 21, she "showed no difficulties with speech, could read a newspaper, and worked as an assistant in a day-care center." Her daughter had presented with delayed language development.

Ring 14, 46,r(14).

This chromosome is prone to ring formation, with over 60 cases reported since one of the earliest reports (Gilgenkrantz et al., 1971). The question of "intact" (i.e., no genes lost) versus deleted ring 14 was addressed in Zollino et al. (2010b), these two types being observed in about one-third and two-thirds, respectively. These authors provide photographs of patients in whom the rings were intact, and with 14q deletions of 0.65 Mb, 1.5 Mb, 2.3 Mb, and 3–5 Mb extent, respectively. No clear genotype-phenotype links could be drawn, albeit that in the least affected child in their series, a child of "low-normal intelligence," the ring was intact. One distinctive feature is a progressive microcephaly, the head circumference at birth typically being normal. Rare cases may present with Prader-Willi-like features (Tzoufi et al., 2007). Given the fact of chromosome 14 being subject to a parent-of-origin effect (p. 361), it was notable that none of these cases had uniparental disomy. A more severe phenotype was seen in one case diagnosed prenatally, in which skeletal anomalies reminiscent of paternal UPD 14 might have been due to loss of the maternal copy of *MEG3* (Quenum-Miraillet et al., 2008). The case in Nucaro et al. (2010) demonstrates the complexity that some rings may show with high resolution studies such as array-CGH.

As for transmission of the ring, Bowser-Riley et al. (1981) described a 46,XX,r(14) mother "at the lower end of the normal range" of intelligence, who had two retarded 46,XX,r(14) daughters (and a third 46,r(14) pregnancy which was terminated).

Ring 15, 46,r(15).

Parent-to-child transmission of 46,r(15) is recorded (Horigome et al., 1992). Glass et al. (2006) illustrate the variability of the r(15) phenotype, contrasting an infant followed up after prenatal diagnosis, who manifested severe growth retardation, dysmorphism and malformations, and marked developmental delay; and an adult woman, employed in a plant nursery, referred for karyotyping for the possibility of Turner syndrome. The infant had a larger distal 15q deletion, and more complex "dynamic mosaicism," compared with the adult (but age at sampling, and tissue variability of ring behavior, prevent clear correlations being drawn); and, applying array-CGH, they could distinguish the respective rings as 15q26.2→qter and 15q26.3→qter. The ring with the larger deletion lacked the growth factor receptor gene *IGFR1*, and this likely contributed to the severe growth restriction in the infant; this association has been reported in a number of other r(15) cases.

The power of microarray analysis is further illustrated in Manolakos et al. (2009), a prenatal diagnostic case in which an apparently balanced r(15) was shown at CVS and further defined at amniocentesis as 46,XX,r(15)(p11.1q26.3)[21]/45,XX,−15[9]. MLPA and 15qter FISH showed no imbalance, but on microarray, a ~500 kb deletion (approx. nt 99,831,000–100,168,000) at distal 15qter was identified; the reader might wish to enter these numbers into the UCSC or Ensembl genome browsers, and observe how

very distal the deletion is, and how few genes (at most, three) are deleted. The fetus was abnormal.

Ring 17, 46,r(17).

Ring 17 may present with the severe neurological picture of Miller-Dieker syndrome (p. 322), if the deletion extends to the *LIS1* locus, or a not quite so severe phenotype with epilepsy, microcephaly, and mental retardation (and café-au-lait macules), if only subtelomeric 17p sequence is removed (Ricard-Mousnier et al., 2007; Surace et al., 2009). Analogous to the ring 22 (see later), somatic loss of the chromosome can be the “first hit” leading to neurofibromatosis type 1 (Havlovicova et al., 2007).

Ring18, 46,r(18).

Consistent features include microcephaly, mental retardation, seizures, maxillofacial dysmorphism, and clefting (Koç et al., 2008; Ono et al., 2010). Stankiewicz et al. (2001a) studied seven phenotypically abnormal cases in some detail. Loss of 18q material was consistent, and thus a picture reminiscent of 18q–resulted, while loss of 18p was variable. The abnormal child in Miller et al. (2003) had two rings, one derived from 18p and the other from 18q.

A mosaic parent can be phenotypically normal but have a high risk to have an abnormal r(18) child (Fryns et al., 1992b). Yardin et al. (2001) document the history of a woman with the ring 18 syndrome, her karyotype 46,XX,r(18)(p11.3q23)[32]/45,XX,–18[4] on peripheral blood analysis. Six pregnancies all had abnormal outcomes, and the three karyotyped also displayed the same mosaicism.

Ring 19, 46,r(19).

Flejter et al. (1996) describe a normal mother having ring 19 mosaicism, 46,XX/46,XX,r(19), with only 4% of cells (lymphocytes) having the ring, while her abnormal daughter was 46,XX,r(19) in 98% of cells. A telomeric probe hybridized to the ring, suggesting a telomere-to-telomere fusion format; and a small ring would be unlikely to undergo dynamic mosaicism, as discussed earlier. Speevak et al. (2003) report a very similar case.

Ring 20, 46,r(20).

Two different cytogenetic forms of this syndrome exist. In most, there is r(20)/normal mosaicism, and a tip-to-tip fusion structure of the ring, of presumed postzygotic origin. In some, there is distal deletion of one or both arms, of likely meiotic origin, and in these cases nonmosaicism is typical, and with a more severe clinical picture (Conlin et al., 2011). Epilepsy is the notable clinical feature (Vignoli et al., 2009). The electroencephalogram (EEG) has a characteristic pattern, with trains of “theta waves.” Any patient with epilepsy who has long runs of epileptiform activity on the EEG in the nonseizing state, which may or may not be associated with confusion or diminished consciousness, should have chromosome analysis with this ring chromosome in mind. Zou et al. (2006) suggest, as one possibility, that silencing of the neuronal channel genes *CHRNA4* and *KCNQ2*, at 20qter, due to a “telomere position effect,” might be the basis of the epilepsy in the tip-to-tip ring.

An otherwise unaffected parent with a lower level of mosaicism can have affected children with the ring chromosome in higher proportion (Canevini et al., 1998). Herrgård et al. (2007) report a mother with 10% r(20) mosaicism, who had an onset of seizures in her mid-twenties, and who was intellectually normal. Her daughter had epilepsy from age 7, and cognitive capacity fell away in subsequent years; her son was always behind in development, showed poor behavior, and had seizures from age 5. These both had 40% of their cells with the r(20).

Ring 21, 46,r(21).

The cognitive phenotypes can vary from normal to mild retardation (Gardner et al., 1986b). The male 46,XY,r(21) heterozygote may be subfertile (Dallapiccola et al., 1986). Falik-Borenstein et al. (1992) report a three-generation kindred. One 46,XX,r(21) heterozygote had had seven pregnancies with four early miscarriages, one normal son, one son with Down syndrome, and one 46,XX,r(21) daughter, the latter herself having a 46,XX,r(21) daughter. Most karyotyped cells in these individuals were 46,r(21), but a few were 45,–21, and some had a double-size or multisize rings. Short stature, but normal IQ/development, accompanied the abnormal karyotype in these females; one male heterozygote may have had a low-normal intelligence. In one baby with 46,XY,r(21)(q22p11.2)[34]/45,XY,–21[4]/46,XY[14] diagnosed prenatally, and normal on assessment at 10 months, the father proved to have one cell out of 100 with the ring, which may have reflected a somatic-gonadal mosaicism (Papoulidis et al., 2010).

Melnyk et al. (1995) discuss the difficulties in counseling, relating to uncertainty of the predicted phenotype, in a three-generation r(21) family in which the (nonmosaic) r(21) persons were of normal appearance and intelligence. A 46,XX,r(21) mother had a prenatal diagnosis that showed one 46,XY twin and the other with 46,XX,r(21)/ 45,XX,–21 mosaicism. Both babies were normal, and the girl's postnatal karyotype was nonmosaic 46,XX,r(21), the same as in the mother. The 45,XX,–21 cell line on amniotic fluid culture may have been of extra-fetal origin or may have arisen as an in vitro artifact. Mother-daughter transmission is recorded in Bertini et al. (2008a), with each having the same karyotype on blood: 46,XX, r(21)/ 45,XX,–21 [98%, 2%]. In this instance, the rearrangement was due to a subtelomeric 21q deletion of 3.4 Mb, and apparently no dosage-sensitive genes had been lost. In their review, Muroya et al. (2002) note other instances of a r(21) parent having a child with a rea(21), possibly indicative of a susceptibility within the ring chromosome to undergo further rearrangement. Their own case illustrated the reverse circumstance: a normal mother with a rather complex der(21) had a mildly mentally retarded son with 46,XY,r(21) (and 4/100 cells 45,XY,–21).

A sperm study on a ring 21 infertile man with an extremely low sperm count, karyotyping 45,XY,–21[3]/46,XY,r(21)[95]/46,XY[2] on blood and with fairly similar proportions on buccal cells, came up with an interesting result: FISH showed most (92%) of 169 spermatozoa to be normal, 7% with the ring, and 1% disomic with the normal 21 and the ring 21. These authors suggested that the (presumed) small fraction in the gonad of normal spermatogonia were selectively favored at meiosis, leading to the majority of gametes being normal (Hammoud et al., 2009).

Ring 22, 46,r(22).

A handful of inherited cases are on record (Teyssier and Moreau, 1985; Crusi and Engel, 1986; Wenger et al., 2000). In some, the ring is inherited from a phenotypically normal parent to phenotypically normal offspring; in other cases, one or more of the family members with the ring have mental retardation or other clinical features. In some cases, the parent is mosaic and the child has inherited the ring in a nonmosaic state, which may partially explain parent-offspring differences in phenotype (Jobanputra et al., 2009). Wenger et al. report an example of parent-to-child transmission. The r(22) mother had required special education at high school. Her son had bowel and heart defects, with very little language development by age 20 months. By a strange coincidence he had, on his other chromosome 22 chromosome, a de novo del(22)(q11.2). Infertility, with complete spermatogenic arrest, is recorded in an otherwise normal man (Zuccarello et al., 2010).

Some ring 22s have a more proximal q arm breakpoint and are deleted for the *ARSA* gene at 22q13; in such cases, the phenotype is essentially that of the 22q13.3 deletion, also known as Phelan-McDermid syndrome (p. 327) (Koç et al., 2009; McGaughran et al., 2010). Denayer et al. (2009) make the point (and nicely illustrate) that café-au-lait macules may be observed in this, and other ring syndromes, and, as noted earlier also with respect to the r(12), that this may mislead to a diagnosis of neurofibromatosis type 1.

A ring 22 may, of itself, function as a “first hit” in the generation of tumors of neural crest origin, due to neurofibromatosis type 2 (NF2). The *NF2* gene is located at 22q12. The neural crest is the embryonic tissue that gives rise to (inter alia) the investing membranes of nervous system structures. Due to its mitotic instability, a cell line in this tissue might lose the ring and thus become monosomic for 22. Subsequently, a mutation occurring in the *NF2* gene on the remaining intact homolog would be “exposed” and allow a classic tumor, a schwannoma of the eighth cranial nerve or a meningioma of the cranial or spinal meninges, to develop (Denayer et al., 2009).

The Supernumerary Small Ring, 47,+(r)

A supernumerary chromosome implies, naturally, a partial trisomy. Daniel and Malafiej (2003) presented six cases of their own and reviewed the literature. Generally, it is

only when the ring chromosome is very small, or when there is mosaicism with a substantial fraction of normal cells—in other words, where the overall load of genetic imbalance is small—that a question of genetic risk for offspring of the heterozygote will be relevant. Postnatally ascertained cases have naturally presented with an abnormal phenotype, but a fraction of cases come to attention fortuitously, some being phenotypically normal. Mosaicism complicates the interpretation. A few cases are known in which a parent with low-level mosaicism has had an abnormal child with a higher proportion of the cells with the ring. Furthermore, the levels of mosaicism as determined from a peripheral blood sample may not necessarily reflect the levels in other tissues, and including brain; and in a number of rings, little correlation is recognized between the degree of mosaicism and the severity of phenotype.

Small supernumerary rings have been reported for every autosome except chromosome 17, as listed in Daniel and Malafiej (2003). Brief sketches of some of these follow, with particular reference to recorded cases in which a parent with the ring has had offspring.

Ring 1, 47,+r(1).

Callen et al. (1999) presented a series of patients with very small supernumerary r(1) chromosomes ranging in phenotype from normal to abnormal and showed that the size of the ring was correlated with phenotype. Mother-to-child transmission is documented in Bernardini et al. (2007), and these authors summarize the literature to that time. They propose that clones RP11–110B10 and RP4–646P11, mapping to 1p and 1q, respectively, can serve as useful boundaries, and if the ring includes additional material extending beyond these points, an abnormal phenotype is to be anticipated. Kosztolányi et al. (2011) report a three-generation family, (normal) grandfather to (nearly normal) mother to two (abnormal) children, this appearance of “anticipation” being actually a reflection of ascertainment bias. Prenatal diagnosis of 47,+(1) is reported (Wray et al., 2007).

Ring 2, 47,+r(2)

A 47,XX,+r(2)/46,XX mother with minor facial dysmorphology and apparently otherwise normal had a son with mosaicism for the same tiny ring chromosome, who presented with mental retardation and a psychotic disorder (Giardino et al., 2002). The ring was present in 54% of cells (peripheral blood) in the mother, and 80% in the son.

Ring 3, 47,+r(3).

A normal mother and her normal infant son had the karyotype 46/47,+r(3), at frequencies of 33% (mother's lymphocytes) and 41% (prenatal diagnosis in the son, amniocyte analysis) (Anderlid et al., 2001).

Ring 4, 47,+r(4).

Bonnet et al (2006) review the ring 4 and describe their own case of a child of low-normal intellect, in whom they demonstrated up to three copies of a very small ring chromosome, about 20 Mb in size, in 82% of cells. Three recorded diagnoses were from amniocentesis; all three pregnancies were terminated, with the very severe brain defect of alobar holoprosencephaly identified in one. In a dramatic display of mosaicism, Soysal et al. (2009) report a case with a 46,XY,r(4)[83]/45,XY,–4[6]/47,XY,r(4),+r(4)[5]/48,XY,r(4),+r(4),+dic r(4)[1]/46,XY[5] karyotype in a patient with cortical dysgenesis, mild mental retardation, seizures, and hip dislocation.

Ring 7, 47,+r(7).

Tan-Sindhunata et al. (2000) report a family in which the mother of low-normal intelligence, and two of her three children, had mosaicism for a very small supernumerary ring, 47,+r(7)/46,N. Although the fractions of mosaicism were similar in the three (about 50%), the children were more severely affected, at least with respect to language acquisition, than their mother. Speculatively, this could reflect, in the mother, a lesser “ring load” in the brain. Her other child was normal. Similar 47,+r(7) cases are recorded in the reviews of Lichtenbelt et al. (2005) and Bertini et al. (2008b); in two, Silver-Russell syndrome was due to UPD 7. The additional copies of the *STX1A* and *LIMK1* genes, common to many r(7) cases, may contribute importantly to the developmental deficits.

Ring 8, 47,+r(8).

The size of the r(8) correlates well with phenotypic outcome, and comprehensive molecular evaluations of r(8) chromosomes would facilitate karyotype-phenotype correlations (Bettio et al., 2008). Daniel and Malafiej (2003) report a normal woman karyotyped because she had had a child with Wolf-Hirschhorn syndrome, and who turned out to have a very small r(8) in 27% of lymphocytes. A phenotype suggestive of the MURCS (Müllerian and renal aplasia, cervicothoracic somite dysplasia) association was seen in the patient of Loeffler et al. (2003), a mildly retarded teenage girl, in whom 70% of cells contained a tiny r(8) chromosome. Filges et al. (2008) studied a developmentally delayed girl mosaic for a small (not tiny) ring 8, and applying array-CGH, they could describe the extent and size of the ring, namely, 47,XX,+r(8)(::p11.21→ q21.2::)[18]/46,XX[12], comprising 43.8 Mb.

Bettio et al. (2008) document a prenatally diagnosed de novo very small ring comprising about 5 Mb of proximal 8p and 8q euchromatin, in mosaic state (50% of cells with the ring on CVS, 90% at amniocentesis, and 96% at postnatal blood sampling). Although early infant development was within the normal range, by age 3 it was clear that language acquisition was poor, and that behavior was affected. A similar case in Gole and Biswas (2005) concerned prenatal diagnosis (amniocentesis and fetal blood) with 50% mosaicism 47,XY,+r(8)(?p11.2q11.2)/46,XY; the child was developing apparently normally at 9 months. However, the level of mosaicism may not necessarily be a useful determinant in prognosis.

Familial transmission is known. A normal father, a university graduate, with low-level mosaicism for a very small supernumerary r(8) had two nonmosaic 47,XX,+r(8) daughters (Rothenmund et al., 1997). They were intellectually handicapped and displayed emotional immaturity, although their physical growth was normal.

Ring 10, 47,+r(10).

Few prenatal diagnoses are on record. Sung et al. (2009) review three reports and describe their own case of mosaic 47,XX,+ r(10)/46,XX detected at amniocentesis and confirmed in the newborn. They extended the study with array-CGH and could thus describe the imbalance as arr cgh 10p11.2q11.2(CN_519687→ CN_541524)×3. The child was normal on assessment at age 1 year.

Ring 12, 47,+r(12).

Rings of chromosome 12 may be of variable makeup: 12p material, 12q, both 12p and 12q, or of uncertain components. No clear clinical phenotype has emerged, other than abnormality in all (Davidsson et al., 2008). Yeung et al. (2009b) document a case in which the ring 12 included two copies of 12p, thus determining a Pallister-Killian phenotype (p. 305).

Ring 15, 47,+r(15).

A exceptional case is that of a small bisatellited supernumerary marker chromosome (SMC) derived from chromosome 15 in grandparent (mosaic) and parent (nonmosaic), evolving into a very small ring 15 in the grandchild. All three, and two other siblings with the SMC, were normal (Adhvaryu et al., 1998).

Ring 18, 47,+r(18).

Jenderny et al. (1993) describe a phenotypically normal mother with 47,XX,+r(18) in only 2/100 cells on blood analysis, the remainder being 46,XX, and who had a daughter with nonmosaic 47,+r(18). A comparable story is reported in Balci et al. (2011) of a mother with a ring in 10% of cells, whose abnormal son was likely conceived as 47,XY,+r(18), but in whom postzygotic events led to his having the mosaic karyotype 46,XY,r(18)/46,XY. A man with a VACTERL-like clinical picture, and with a normal

intellect, carried at low-level mosaicism a r(18) that endowed "octasomy" for a ~5 Mb segment of pericentromeric chromosome 18 (van der Veken et al., 2010).

Ring 20, 47,+r(20).

Guediche et al. (2010) provide a review of 13 cases, eight ascertained postnatally and five prenatally, with psychomotor and growth retardation as frequent, but not universal observations. Kitsiou-Tzeli et al. (2009) document prenatal diagnosis, following which the child, at age 3 months, was judged to be essentially normal; in contrast, Callier et al. (2009) describe prenatal diagnosis based upon array-CGH, in which the aborted fetus displayed dysmorphic features. These several authors point to the need for precision in the molecular delineation of the ring, to enable a clearer genotype-phenotype correlation (a common plea that could of course apply to any of the ring chromosomes). We know of no adult cases.

Ring 22, 47,+r(22).

Mears et al. (1995) document a family in which a phenotypically normal grandfather and father were mosaic for a tiny ring 22 chromosome, 47,XY,+r(22)/48,XY,+r(22),+r(22). A grandchild, also 47,+r(22)/48,+r(22),+r(22) but whose ring chromosomes had increased in size, had cat-eye syndrome (see p. [link]).

Rare Complexities

Supernumerary Ring with a Balancing Deletion.

If a ring chromosome is derived from a segment of chromosome that has been deleted interstitially from an autosome, and if this newly generated ring contains a centromere, it can, in some cases, be transmitted stably at mitosis; and, if so, the karyotype is balanced (p. 158). But the carrier can be at high risk to produce unbalanced gametes. The ring might be transmitted as a supernumerary chromosome, to give a partial trisomy. Or the normal homolog might be replaced by the deleted chromosome, for a partial monosomy. And, even if it is the balanced combination that is present at conception, a substantial risk exists for postzygotic mosaicism (which might or might not generate an abnormal phenotype).

If the ring is very small, the balancing deletion may be missed on classical cytogenetics, as Baldwin et al. (2008) describe in a mother whose karyotype, at first sight, was 47,XX,+r(4)/46,XX; but the small r(4) was in fact derived from a deleted segment of 4p on one of her chromosome 4 homologs. Her child, who inherited this small ring, but not the balancing deleted 4, had a "mild speech delay." Mantzouratou et al. (2009) studied embryos from a couple, the wife being 47,XX,del(22),+r(22), and herself normal. They had had two natural pregnancies, both mosaic 47,+r(22)/46, the first producing an abnormal child, and the second terminated after prenatal diagnosis. Unfortunately, following two preimplantation genetic diagnosis (PGD) cycles, none of the embryos had received the normal, intact maternal chromosome 22, and thus none were transferred.

Formation of a Neocentromere.

A fragment of a chromosome not containing a centromere would not normally be able to be transmitted during cell division. But if a "neocentromere" is generated (p. 307), its survival may be assured; and again we may be dealing with a supernumerary ring that balances a deletion. Slater et al. (1999) describe such a scenario in an infertile but otherwise normal man. A segment was deleted from one chromosome 1, and this same segment (1p32p36.1) existed as a tiny supernumerary ring chromosome. This man thus has the karyotype 47,XY,del(1)(p32p26.1)+r(1)(p32p36.1). The ring chromosome was able to activate the formation of certain centromere binding proteins, which presumably enabled its stable transmission. A similar circumstance is recorded in Knegt et al. (2003), in this case a phenotypically normal woman who had presented with recurrent miscarriage, and in whom a tiny ring 13 chromosome was derived from an interstitial deletion of the segment 13q21.31-q22.2. Amniocenteses in her fourth and fifth pregnancies demonstrated normal karyotypes.

More Than One Ring.

Multiple tiny rings were identified in a unique case (Vermeesch et al., 1999). In this retarded male, fibroblasts culture showed four tiny rings per cell, and in lymphocytes, six. Each ring had a functional centromere. The formation of supernumerary rings would be expected to be a sporadic event, unrelated to any particular predisposition. Very rare reports might belie that supposition as a universal truth. Callen et al. (1991) recorded two retarded children each with two supernumerary small rings: one with a r(6) and an r(X), and the other with an r(3) and another small unidentified ring. Shanske et al. (1999b) described monozygous twins both had two very small supernumerary rings, with similar proportions of cells in each child: about 60% with 48,XX,+r(1),+r(16), 30% with either 47,XX,+r(1) or 47,XX,+r(16), and 10% with 46,XX. Although too small to be confirmed as ring chromosomes, Ballif et al. (2007b) reported a patient with two sSMCs, one derived from 11q, and one derived from 17p.

Genetic Counseling

Parental Karyotype 46,(r)

The great majority of transmitting parents are 46,XX,(r) mothers, presumably reflecting that most male heterozygotes are infertile. The observed risk for the 46,(r) parent to have a child with the same karyotype is a little less than the theoretical 50%, and a figure of about 40% will generally be a fair one to offer. Those offspring inheriting the ring could be expected to present the similar clinical picture as, and indeed quite probably more severely than, their heterozygous parent. In the review of Kosztolányi et al. (1991), about one-third of 46,(r) children were more severely affected mentally than their parent. The 46,(r) parent may be an atypical ring carrier, perhaps with a fortunate pattern of mitotic disruption, to have reached the level of social phenotype that procreation would be likely.

In the particular case of the 46,r(21) heterozygote, who is often phenotypically normal, there is a small but as yet unquantified risk of having a child with Down syndrome due to an uncommon karyotype: 47,+r(21), 46,rob(21q;21q) or 46,tan dup (21q;21q) (Kosztolányi et al., 1991). If, in prenatal diagnosis for a pregnancy of a r(21) heterozygote parent, the same r(21) karyotype were demonstrated in the fetus, based on the slender evidence thus far available, the chance for phenotypic normality would seem to be "substantial," but a (probably mild) degree of abnormality can by no means be excluded. As Kennerknecht et al. (1990) comment "accurate phenotype-karyotype correlations cannot be made, since there are carriers with a stable ring chromosome who are affected, whereas others with an unstable ring have a normal phenotype and vice versa."

In a person who is mosaic on somatic analysis, with a 46,N/46,(r) karyotype, the mosaicism might extend also into the gonad. This would convey an important risk to have a nonmosaic 46,(r) child.

Parental Karyotype 47,+(r)

Each ring needs to be assessed individually, and careful cytogenetic analysis is urged. Reference to the brief outlines earlier will give a sense of the range of outcomes. A nonmosaic parent with a very small ring might be expected to transmit the abnormal chromosome with 50% probability, assuming (and this may not necessarily be the case) meiotic and mitotic stability. The parental phenotype would, in principle, predict that of the 47,+r child. Mosaicism in the parent, and potential mosaicism in the child, considerably complicate prediction. A higher-grade mosaicism in the child than in the parent, or complete nonmosaicism in the child, would be expected to produce a more severe phenotype, possibly lethal in utero.

Parental Karyotype 47,del(A),+r(A).

In the ring with a balancing deletion (see earlier), normality in an offspring can only be regarded as secure (other things being equal) in the context of the normal homolog (A)[1] having been transmitted from the 47,del(A),+r(A) parent. Even though the carrier parent may be normal, the risk is high that the same balanced karyotype in a conceptus could be followed by postzygotic misdivision, with the eventual generation of offspring who would be partially trisomic, or partially monosomic, for the autosome concerned, and

thus abnormal. A detailed discussion is offered in Mantzouratou et al. (2009).

Notes:

[1] "A" indicates any autosome.



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Complex Rearrangements

Chapter: Complex Rearrangements
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COMPLEX CHROMOSOMAL REARRANGEMENTS (CCRs) occurring in phenotypically normal persons are rare. Familial cases comprise a minority, with only about 35 examples recorded by the early 2000s (Berend et al., 2002b). Three or more chromosomes are involved, and a considerable variety of rearrangements are possible. Translocation may involve distal segments, as in the usual reciprocal translocation, or interstitial segments, as in the insertion. An inversion and a translocation, for example, may coexist on the same chromosome.

Biology

Three Major Categories of Complex Chromosome Rearrangement

Three major categories of CCR are recognized (Kausch et al., 1988). The most common is the *three-way exchange*, in which three segments from three chromosomes break off, translocate, and unite (Fig. **12–1**). Most three-way CCRs are familial, usually transmitted through the mother; although in one of the largest kindreds on record, showing five-generation transmission, three (great)grandfathers must have been CCR heterozygotes (Farrell et al., 1994).[1] More complicated, *exceptional CCRs* encompass a wide theoretical range, but there are not many actual cases. (These two types are also referred to as CCR types I and II.) The simplest CCR is the *double two-way exchange*, in which there is a coincidence of two separate simple reciprocal translocations. In a sense, the double two-way exchange is not a "true" CCR, and it might as well be described as a double or a multiple rearrangement (Phelan et al., 1990). Similarly, there may be the coincidence of a reciprocal translocation with a Robertsonian translocation, or with an inversion.

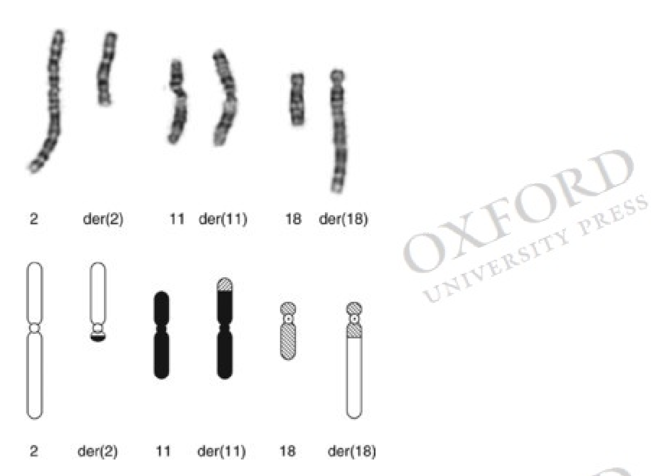




Figure 12–1
A three-way complex chromosome rearrangement. Most of 2q is translocated onto 18q; part of 18q is translocated onto 11p; and the tip of 11p is translocated onto 2q. The individual had presented with multiple miscarriages. (From R. J. M. Gardner et al. (1986a), A three way translocation in mother and daughter, *Journal of Medical Genetics* 23:90. Reproduced with the permission of the British Medical Association.)

An apparently balanced karyotype may be associated with a normal or an abnormal phenotype. If the individual is phenotypically normal, the chromosome rearrangement is assumed to be truly balanced. These cases are often familial. The original CCR in a family typically arises as a single complex event, rather than sequential changes, at a meiosis during male gametogenesis (Grossmann et al., 2010). In the phenotypically abnormal individual, presumably some submicroscopic imbalance or other genetic defect exists, and these cases characteristically involve a de novo chromosome abnormality. Such a case was proven in a CCR studied by Brandt et al. (1997), in a child with tricho-rhino-phalangeal syndrome (TRPS; see p. [link]). The child had a de novo apparently balanced t(7;13;8)(p21;q21;q24.1), but using fluorescence in situ hybridization

(FISH) probes for the TRPS critical region at 8q24.1, a 3 Mb deletion was revealed. In a systematic microarray-based study of 13 abnormal individuals, two normal women who had had recurrent pregnancy loss, and three prenatal cases, De Gregori et al. (2007) showed imbalances in all except one of the abnormals, and one of the normal women. The imbalances comprised microdeletions, in some just a single one, in some two, and in two patients, three and four microdeletions, respectively.

Details of Meiotic Behavior

The carrier of a CCR has a risk for an abnormal conception due either to malsegregation of the derivative chromosomes or to the generation of a recombinant chromosome. Malsegregation follows the general principles as set forth for the simple translocation, but naturally the range of unbalanced combinations is greater. For the three-way CCR, the broad categories of malsegregation are 3:3 and 4:2, and (theoretically) 5:1, and 6:0. Recombination, whether producing a balanced or unbalanced karyotype, is rare indeed, and only eight such familial CCRs were recorded in the review of Berend et al. (2002b). In the exceptional CCR, scarcely ever are meiotic recombinants observed, and the family in Gruchy et al. (2010), in which a CCR with five insertional translocations with eight breakpoints was transmitted over three generations without recombinant offspring, is typical in this respect.

Three-Way Complex Chromosome Rearrangement

At meiosis in the three-way CCR heterozygote, the expectation is that the chromosomes involved in the rearrangement will come together and form a multivalent (Saadallah and Hultén, 1985; Fig. **12–2**). Consider how meiosis would proceed in the rcp(2;18;11) translocation illustrated in Figure **12–1**. In theory, a hexavalent configuration would allow full synapsis of homologous segments (Fig. **12–3**). If disjunction were then symmetric (3:3), up to 20 possible gametic combinations could occur. The two arising from alternate segregation (arrows in Fig. **12–3**) would be the only ones to be balanced; the remaining 18 would be unbalanced to a greater or lesser degree. Were asymmetric segregation (4:2, 5:1, 6:0) to occur, a great variety of extremely unbalanced gametes would result. However, it may be that, in some families at least, a tendency to favor symmetric alternate segregation, and a combination of very early lethality of severely unbalanced conceptuses, imply a fair prospect for achieving a normal pregnancy (Walker and Bocian, 1987). An excess of heterozygotes has been noted among the balanced female offspring (Batista et al., 1994).

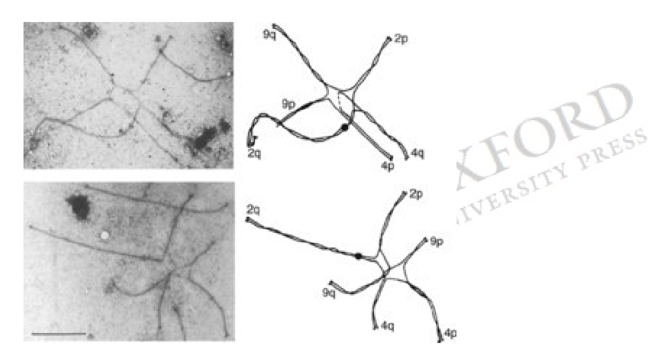




Figure 12–2
The actual appearance of a multivalent at meiosis I. Electronmicrograph of a spermatocyte from a testicular biopsy of a man with a three-way complex chromosomal rearrangement 46,XY,rcp(2;4;9)(p12;q25;p12); line drawing shows component parts of the hexavalent. From N. Saadallah and M. Hultén, 1985, A complex three breakpoint translocation involving chromosomes 2, 4, and 9 identified by meiotic investigations of a human male ascertained for subfertility, *Human Genetics* 71:312–320. Courtesy MA Hultén, and reproduced with the permission of Springer-Verlag.)

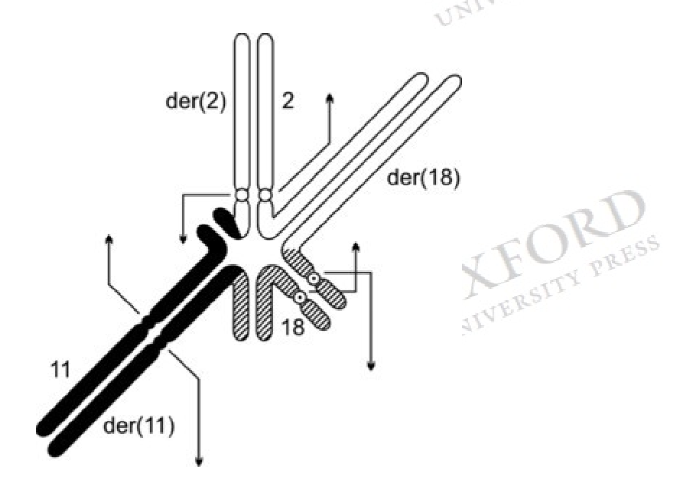




Figure 12–3
Diagrammatic representation of the formation of a hexavalent at meiosis in the three-way 2;18;11 translocation depicted in Figure **12–1**. The arrows indicate 3:3 alternate segregation.

The risk of having a pregnancy that would go to term but produce an abnormal child reflects the nature of the rearrangement—that is, whether there are possible chromosomal combinations that would lead to aneuploidy for a survivable amount of genetic material. Thus, considering the preceding rcp(2;18;11) example, three unbalanced combinations, one 3:3 and two 4:2, might be expected to be viable (Fig. **12–4**). Batista et al. (1994), reviewing 29 families with a CCR, determined that an abnormal live birth is most commonly (78%) due to 3:3 adjacent-1 segregation, followed by 4:2 segregation. Recombination would add yet further possibility of imbalance, but this is, as mentioned earlier, very rarely seen.

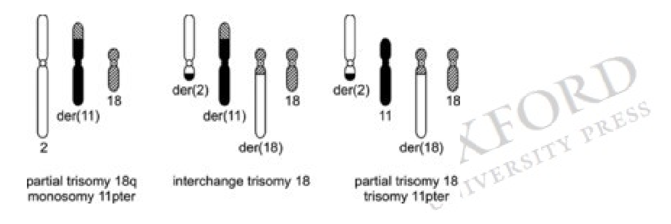




Figure 12–4
Three segregant outcomes of meiosis in the rcp(2;18;11) heterozygote shown in Figure **12–1**, that might be expected to produce viable but unbalanced offspring. The 3:3 adjacent-1 gamete on the left may be the one most likely to be produced.

The concept of adjacent-1 and adjacent-2 segregations can be applied in the setting of the CCR, in the case of 3:3 segregations. Thus, the segregant gamete shown at left in Figure **12–4**, having one of each chromosome pair represented (one of each centromere), would reflect adjacent-1 segregation. An example of 3:3 adjacent-2 segregation is given in Xu et al. (1997). A mother had the karyotype 46,XX,t(5;16;22), and cytogenetic analysis of her morphologically abnormal fetus following intrauterine death at 16 weeks gestation showed 46,XY, der(5),der(16),−22,t(5;16;22). In this case, the abnormal ovum would have had one chromosome 5, two chromosome 16s (one normal, one the derivative), and lacked a chromosome 22.

4:2 segregation particularly characterizes CCRs in which an acrocentric chromosome is a component. Schwinger et al. (1975) reported a mother of two children with typical Down syndrome, who herself had a three-way t(7;21;11) CCR. The affected children had an interchange trisomy 21, in that they had, in addition to the maternal translocation pattern, a second intact chromosome 21. Fuster et al. (1997) give an example of a 4:2 malsegregant, diagnosed at chorionic villus sampling, from a three-way paternal t(2;22;11) CCR. The fetal karyotype was interpreted as 47,−2,der(2),der(22)t(2;22;11)(q13; q11.2;q23). The parents continued the pregnancy, and the retarded and abnormal child had a double partial trisomy: a duplication of the segments 11q23-qter and 22pter-q11.2. The couple had previously had one normal child and three miscarriages.

Exceptional Complex Chromosome Rearrangement

More complex rearrangements imply an even greater potential range of abnormal gametes. Kausch et al. (1988) calculated a minimum of 70 possible unbalanced gametes due to 4:4, 5:3, 6:2, and 7:1 segregations from an octavalent, in the case of a woman with a five-breakpoint CCR with translocations of chromosomes 1, 2, 5, and 11 and an inversion of chromosome 1, who had presented with three first-trimester miscarriages. Van der Burgt et al. (1992) report a similarly complex de novo balanced CCR (chromosomes 5, 11, 12, 16; five breakpoints in all) in a mother who had had one miscarriage, one 46,XY child, the index abnormal child, and, as a quite unexpected outcome, a de novo 45,rob(13q14q) at prenatal diagnosis in her fourth pregnancy.

One of the most complicated familial CCR scenarios ever described is the case in Röthlisberger et al. (1999). A father carried a de novo rcp(6;7; 18;21)(q22 and q25;q21.3, q31.1 and q32.1;p11.21 and q21.3;q21.3). As this unofficial nomenclature attempts to indicate, there were eight breakpoints altogether, two in chromosomes 6 and 18, three in 7, and one in 21. FISH and spectral karyotyping were needed to clarify the detail of the rearrangement. Most remarkably, among his three children, three different recombinant forms were passed on: a rec(7), a rec(21), and a rec(18). The child with the rec(21) had a balanced karyotype, and he has become a balanced carrier for a simple translocation, 46,t(7;21)(q21.3;q21.3): a “rebuilt” translocation (see later). The other two have partial trisomies for 6q and 7q.

A subtler example is the case in Gibson et al. (1997) of a mother with a de novo five-break rearrangement in which two small interstitial segments in 2q and 5q, and two terminal segments in 1q and 5q, exchanged position. Her abnormal child was initially thought to have a possibly unbalanced t(1q;5q), but further analysis revealed his wonderfully complicated true karyotype, and the reader may care to draw an ideogram and then compare with the original paper:

46,XY,der(1),der(5),t(1;5;2)(1pter→1q42.3::

5q23.2 →5qter;5pter→5q21.2::

2q33→2q35::1q42.3→1qter;2pter→2q33::

5q21.2→5q23.2::2q35→2qter)mat.

An unbalanced CCR could be “corrected” by having the countertype imbalance in another rearrangement. Thus, the mother in Zou et al. (2010) had a three-way CCR t(5;15;7)(q13;q24;p15), which was missing the segment 5q13.1-q14.1. But this segment was otherwise present, as an insertion into a chromosome 4, as der(4)ins(4;5)(q31.3;q13. 1q14.1), thus qualifying as an exceptional CCR. She was phenotypically normal and (unsurprisingly, therefore) balanced on microarray analysis. However, she transmitted this der(4) to her son, whose karyotype was 46,XY,der(4)ins(4;5)(q31.3;q13.1q14.1)mat, and he presented an abnormal clinical picture, due to this segmental duplication. In molecular nomenclature, the imbalance is arr 5q13.1q14.1 (66,783,672–77,559,998)×3 mat, representing a 10.8 Mb duplication.

Cryptic Complex Chromosome Rearrangement

A CCR may be shown, upon detailed cytomolecular study, to have a greater number of breakpoints or more complex imbalance than had originally been appreciated (Batista et al., 1994; Ballarati et al., 2009); and it may be a little arbitrary as to whether it is categorized as a cryptic or an exceptional CCR. From the days of classical cytogenetics, Wagstaff and Hemann (1995) describe a phenotypically normal father and his two abnormal children, the father and son having an apparently balanced 46,XY,rcp(3;9)(p11;p23) and the daughter apparently 46,XX. On FISH and DNA studies, they could show that the father had a tiny segment of chromatin from the breakpoint in 9p23 removed and inserted into the long arm of a chromosome 8 (Fig. **12–7**). At meiosis, it may have been that a quadrivalent formed from the chromosome 3 and chromosome 9 elements, while the two chromosome 8 homologs synapsed independently as a bivalent. On this interpretation, the two children reflect alternate segregation of the chromosome 3 and 9 elements; with respect to the homologs of chromosome 8, the rcp(3;9) son inherited his father's normal homolog, and so the lack of the 9p23 segment was not corrected, while the “46,XX” daughter received the chromosome 8 with the 9p23 insertion. Thus, the son has a del(9)(p23), and the daughter a dup(9)(p23).

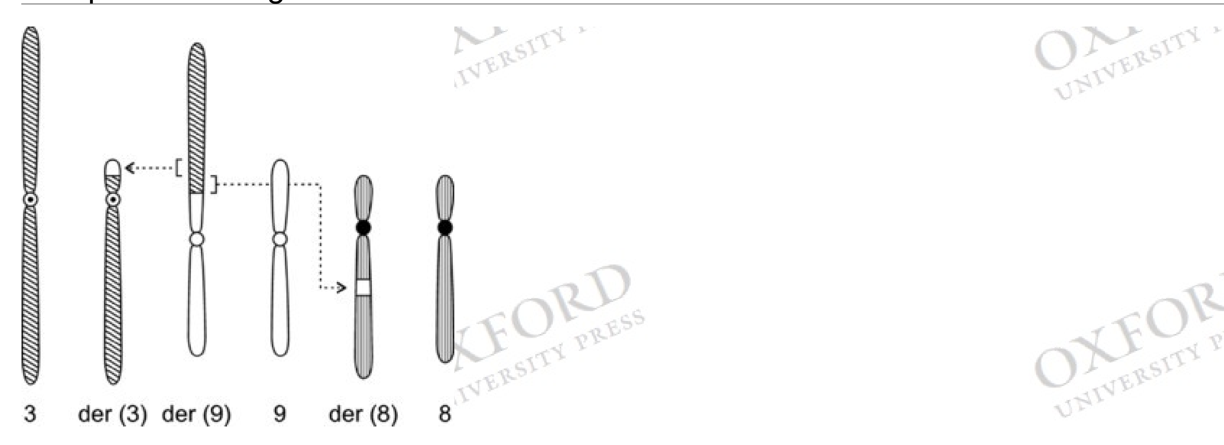


Figure 12–7
A cryptic complex chromosome rearrangement (and see text). On the original cytogenetic study, father and son appeared to have the same simple balanced translocation, 46,XY,rcp(3;9)(p11;p23), and the daughter seemed to be 46,XX. DNA and fluorescence in situ hybridization (FISH) studies showed a complex chromosomal rearrangement, in which a tiny segment within 9p23 had been insertionally translocated into 8q in the father. Brackets and dotted lines show translocation of two separate segments from distal 9p across to 3p and to 8q respectively. Thus, both the son and the daughter had an unbalanced complement, the son with a deletion, and the daughter with a duplication, for the 9p23 segment. (From the family reported in Wagstaff and Hemann, 1995.)

Further insight may come from the application of newer methodologies. For example, in a study of two previously diagnosed cases of de novo CCR, identified in phenotypically abnormal individuals, de Vree et al. (2009) undertook array comparative genome hybridization (CGH) and M-FISH analyses. In the first case, a teenager, the original karyotype of 46,XY,t(1;18;15)(q32;q21;q24) could be shown to include, as well, a 1.5 Mb microdeletion at 10p13: the full karyotype could now be rewritten (the italics are ours) as

46,XY,der(1)(1pter→1q31::

10p14→10pter),der(10)(15qter→15q24::

10p13→10qter) *del(10)(p13p13)*,der(15)(15pter→

15q24::18q21→18qter),der(18)(18pter→18q21::

10p13→10p14::1q31→1qter)dn.

In the second case, a severely retarded adult, the original karyotype was 46,XY,del(5)(q11),der(11)t(5;11)(q11;q11),der(13)t(11;13)(q11;p11). On modern reanalysis, the rearrangement was seen to be more complex, and the 5q11 deletion not to exist:

46,XY,der(5)(5pter→5p10), der(9)(9pter→9q31::

5q31→5q31::

13q31→13q31::9q31→9qter),der(11)(13qter→13q31::5q31→5q10::

11q10→11qter),dic(11;13)(11pter→11p10::

13p13→13q31::5q31→5qter)dn.

In spite of this complexity, on array-CGH no actual pathogenic genomic imbalance was recognized. Thus, other reasons needed to be invoked (e.g., position effect) as possible causes of the abnormal physical and cognitive phenotype.

A familial case in which a supposed simple translocation was shown to be a CCR, and then with a microdeletion coming to light on molecular analysis, is presented in Aboura et al. (2003). A mother and her infant son, the latter with minor dysmorphism and abnormal functional neurology, appeared at first to have the same simple t(3q;22q) translocation. FISH analysis showed this to be a t(3;22;9)(q22;q12;q34.1).Yet finer analysis using a probe to the *ABL* locus on 9q34.1 revealed a very small deletion at this site on the der(9) of the proband, but not in his mother or in a carrier sister. The deletion was thus presumed to have arisen de novo, during maternal meiosis. These scenarios raise pressing questions: how often might other apparently balanced simple reciprocal translocations have a cryptic complex rearrangement; and how often does a de novo deletion occur on the background of a parental balanced rearrangement (see p. [link])?

Double Two-Way Complex Chromosomal Rearrangement

Presumably, two separate and independently operating quadrivalents can form (Bowser-Riley et al., 1988). Burns et al. (1986) record sperm karyotypes in a man with a double two-way CCR 46,XY,rcp(5;11)(p13;q23.2),rcp(7;14)(q11.23;q24.1), whose wife had had four miscarriages, a child with cri du chat syndrome, and a normal son carrying the rcp(7;14). Only four of 23 sperm analyzed had an overall balanced complement, and the majority (13) had adjacent-1 segregants for one or the other translocation. Another five showed 3:1 and one sperm showed 4:0 segregation.[2]

We referred on p. 97 to a couple in which both had a simple reciprocal translocation, and both happened to involve chromosome 7 (7p in one, 7q in the other). It is a useful exercise to imagine how the chromosomes might be transmitted in this family. The couple could, in theory, have a child with a double two-way CCR who would have a combination of their own karyotypes. Providing fertility were not compromised, this child of theirs in generation II could then, in generation III, have two types of balanced progeny: one with the rcp(7;11), and the other with the rcp(7;22), as in the couple of generation I. We set out this scenario in Figure **12–6**. No offspring with a normal karyotype could be produced in generation III, unless recombination between the two der(7) chromosomes were to restore a normal chromosome 7.

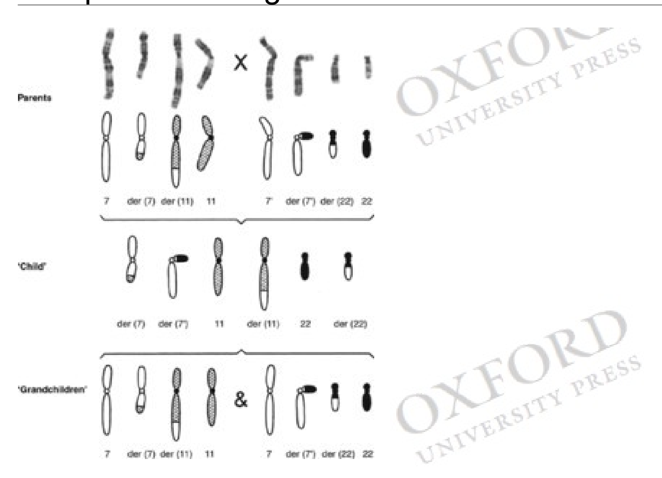




Figure 12–6
Theoretical potential pedigree of a couple each of whom carries a simple balanced reciprocal translocation: 46,XY,rcp(7;11)(q22;q23) and 46,XX,rcp(7;22)(p13;q11.2). A child of theirs could have a double two-way complex chromosomal rearrangement combining the two parental karyotypes: 46,rcp(7;11)(q22;q23)rcp(7;22)(p13;q11.2). The original simple translocation karyotypes could be restored in the next generation. The reader can determine how, following one recombination, a 46,N grandchild could be conceived. (Courtesy K. L. Butler.)

"Rebuilding" of Chromosomes from a Parental Complex Chromosomal Rearrangement

The coming together of several translocation chromosomes during meiosis may set the stage for what Soler et al. (2005) describe as "rebuilding." The CCR shown in Figure **12–5** with six breakpoints in five chromosomes offers useful illustration (Bass et al., 1985). The woman who carried this rearrangement had four pregnancies, only one of which miscarried, and two produced offspring with a balanced constitution, though different in each child and different from their mother! Recombination involving the centric segment of chromosome 1 led to a daughter receiving a rebuilt der(1), with just the 6p segment being translocated, and a son with a different rebuilt der(1) having just the 7q segment. A son and a grandson had unbalanced karyotypes, which were different, but each led to partial 7q trisomy. Readers who relish esoteric puzzles may wish to refer to the original paper. Rebuilding can lead to a simpler rearrangement. Madan et al. (1997) describe a mother with a familial four-breakpoint t(2;3;8) in which the 2q translocated segment had split with 2q23-q33 going to the der(3) and 2q33-qter to the der(8). Her child's karyotype was a simple 46,t(2;3) with a rebuilt der(3); the simplification resulted from recombination at maternal meiosis between her der(3) and normal chromosome 2. A similar story is told in Tihy et al. (2005).

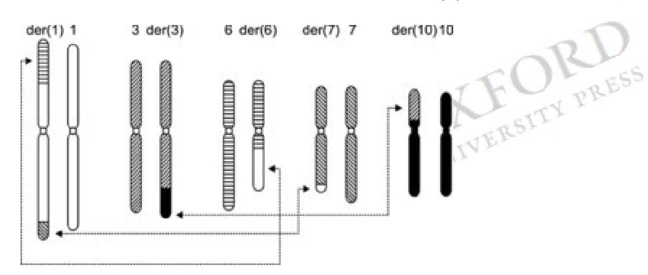


Figure 12–5
An extraordinarily complex rearrangement involving three two-way exchanges, with six breakpoints in five chromosomes (see text). (From the family reported in Bass et al., 1985.)

A unique case of rebuilding in sequential generations is given in Zahed et al. (1998). A grandfather had two separate translocations: a simple translocation rcp(1;8)(p31;q21.1) and an insertional translocation ins(9;8)(q34;p23.1pter). Thus, he had two abnormal no. 8 chromosomes, one having a segment from distal 1p attached at 8q21.1, and the other having a deletion at 8p23.1. He had a daughter and a son to each of whom he transmitted a rec(8), the same rec(8) to each, in balanced state. This rec(8) had a deletion at its p extremity, and a 1p translocated segment on its q extremity. Presumably, his two abnormal no. 8 chromosomes had recombined in meiosis, at a point somewhere between the p23.1 and q21.1 breakpoints. His daughter in turn had two children, and in each of them she restored, by recombination again in this generation—a rebuilt rebuilding!—the grandpaternal chromosomes: the del(8p) in one child, and the der(8)t(1p;8q) in the other. Both children had an unbalanced state, but different in each. One had a straightforward del(8)(p23.1) karyotype, and thus a partial 8p monosomy. The other had the grandpaternal simple rcp(1;8) and would otherwise have been normal; but in addition she inherited the ins(9), which conferred a partial 8p trisomy. The reader may care to draw the chromosomes of the three generations from this description and check back to Figure 2 in the original paper.

Effect upon Fertility

In several complex rearrangements, in the female at least, gametogenesis can accommodate itself to the complexity thrust upon it, and the heterozygote may be fertile and have pregnancies that produce phenotypically normal children. However, the rule of the greater vulnerability of spermatogenesis to chromosomal complexity seems to apply particularly in the situation of the CCR, and the male heterozygote is often sterile due to spermatogenic arrest, or subfertile (Lee et al., 2006; Bartels et al., 2007; Ergul et al., 2009). The involvement of an acrocentric chromosome in the CCR may particularly predispose to this male sterility (Gabriel-Robez et al., 1986). Fertility treatment with intracytoplasmic sperm injection (ICSI) may enable fatherhood (Joly-Helas et al., 2007). Having made these points, a few CCR men do retain natural fertility (Cai et al., 2001).

Genetic Counseling

The male CCR heterozygote who is not otherwise known to be fertile should have a semen analysis to check whether sperm are being produced. If there is oligospermia, IVF will have to be considered, and preimplantation genetic diagnosis (see later discussion) may be appropriate. For the heterozygote (male or female) who is fertile, or for whom fertility can be achieved, a conceptus having either a normal chromosome constitution, or the same balanced CCR as the parent, would be expected to produce a normal child. But a high proportion of conceptions have an unbalanced karyotype. Madan et al. (1997) have determined empiric risk estimates. Overall, the risk for spontaneous abortion is 50%, and the risk for a liveborn abnormal child is 20%. The level of risk is related to the mode of ascertainment—whether through the birth of abnormal infants, multiple miscarriage, male infertility with abnormal spermatogenesis, or fortuitously—and to the family history. If multiple miscarriages have been the pattern in the family in the past, it is likely to continue to be so. In such cases, it may be that all unbalanced forms would lead to miscarriage (Creasy, 1989). If abnormal infants have been born, carriers are

likely to have a high risk for the same unfortunate event to happen again.

For the three-way CCR, it is generally justifiable to advise that, sooner or later, a normal outcome could possibly be expected. Thus, the couple may be willing to make continued attempts until a successful pregnancy is achieved. As always, the pedigree should be studied, in order to understand what might be the particular pattern of meiotic behavior with that CCR. If the reproductive history is very unpromising, optimism may need to be guarded, and the reality of a low chance for a normal child faced (Evans et al., 1984). As for the exceptional CCR, the likelihood for a successful pregnancy would be less, and possibly very small.

Bowser-Riley et al. (1988) review the specific case of the double two-way translocation and propose that the risk to have an abnormal child would be approximately the sum of the figures derived separately for each rcp. They acknowledge that might be an overestimate due to nonviability of doubly imbalanced combinations, albeit each on its own might be viable.

Prenatal Diagnosis

Once a pregnancy is actually achieved, some may prefer initially to rely on first-trimester ultrasonography, declining chorionic villus sampling, and leaving early abortion to happen naturally if that would be the case, as an unfortunate previous miscarriage history might well cause a heightened sensitivity to the small risk associated with prenatal diagnosis. Others may prefer the early information that a chorionic villus sampling could provide. If the pregnancy continues normally by ultrasound criteria into the second trimester, a judgment can be made whether this of itself would be sufficiently reassuring (perhaps in the setting of all unbalanced forms being very unbalanced), or whether amniocentesis would in fact be desirable. On several levels, each case will have to be assessed on its merits. The CCR will need to be very carefully characterized cytogenetically in the parent and the fetus to ensure accurate prenatal diagnosis; chromosomal microarray is likely to command an increasing role in this setting.

The same balanced state identified at prenatal diagnosis raises the same questions, but more pointedly, as in the simple reciprocal translocation (p. 109). By way of example is the CCR 46,XX,t(5;16;10;18)(q13;q22;q11.2;q21) identified at routine prenatal diagnosis in a woman having a history of recurrent miscarriage, reported in Lee et al. (2002), with the same karyotype then being shown in herself. Normal ultrasonography was encouraging, and the pregnancy was continued; at age 2 years, the child was normal. But this fortunate outcome could not have been “guaranteed.”

With an exceptional CCR, interpretation may be aided by array-CGH. Malvestiti et al. (2010) identified a CCR at amniocentesis, following the discovery of multiple fetal malformations at ultrasonography (which naturally gave a strong indication that the karyotype would be unbalanced). The fetal karyotype was interpreted to be 46,XY,der (4)ins(1;4)(q25;q25q31.1), due to a maternal CCR 46,XX, der(1)ins(1;4)(q25;q25q31.1)t(1;5)(q41;q35),der 4)ins(1;4),der(5)t(1;5). Proceeding to array-CGH, the (normal) mother's genome was balanced, but a fetal deletion at 4q27q31.23, a gene-rich region of about 30 Mb, could clearly be appreciated.

Prenatal detection of a de novo CCR is discussed on p. 468.

Preimplantation Genetic Diagnosis

Given the very high fraction of embryos expected to be chromosomally unbalanced, preimplantation genetic diagnosis (PGD) would have an obvious attraction, in order to select in favor of the few embryos, if such there be, that might be normal or balanced. We have seen a couple, the husband having a double two-way CCR of karyotype 46,XY,t(2;20)(p25.1;p11.23),t(4;8)(q27;p21.1), who had presented following four first-trimester miscarriages, although their first pregnancy having produced a normal (but unkaryotyped) son. Of 320 theoretically possible karyotypes, only four (1¼%) would be balanced (and thus raising a glimmer of hope that their first fortunate pregnancy might reflect a tendency toward a balanced combination). It was a challenge for the laboratory to develop a sequential FISH strategy, to cover all the possibilities. In the event, following ovulation stimulation with the collection of 25 eggs, of which 23 were subjected to ICSI and 18 embryos resulting, biopsy was achieved in 15 embryos; but none had a balanced constitution.

A happier outcome attended the efforts of Escudero et al. (2008) and Lim et al. (2008a), these two groups providing PGD to some eight couples carrying a range of types of CCR: three-way, double two-way, reciprocal plus insertion, reciprocal plus Robertsonian, and others. Four of the couples eventually had a take-home baby. The babies were outnumbered by the created embryos 50 to 1, attesting to the very high genetic risk conveyed by the CCR.

Notes:

[1] In the ISCN description of the karyotype, the order of chromosomes in the three-way CCR is as follows: first, the lowest number (or X) chromosome; second, the chromosome that receives a segment from the first; and last, the chromosome donating a segment to the first listed chromosome. Thus, the karyotype for the CCR shown in Figure **12–1** is written 46,XX,t(2;18;11)(q13;q21.1;p15.3).

[2] This case is instructive in illustrating the point that different rcps can have different meiotic behavior: for example, 60% of the rcp(5;11) segregants but only 30% of the rcp(7;14) showed alternate segregation, the environment accounted for by both translocations acting in the same gonad.







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Chromosome Abnormalities and Genetic Counseling (4 ed.)

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Parental Sex Chromosome Aneuploidy

Chapter: Parental Sex Chromosome Aneuploidy
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THERE ARE FOUR MAJOR sex chromosome abnormalities. Otherwise unassisted, infertility is practically inevitable in XXY Klinefelter syndrome and 45,X Turner syndrome, and almost always in Turner variants. The other two conditions, XXX and XYY, apparently have little effect on fertility; furthermore, they are not discernibly associated with any increased risk for chromosomally abnormal offspring. Mosaic forms need to be considered on their own merits.

Biology

We need briefly to consider why X chromosome aneuploidy is associated with so little phenotypic abnormality, compared with autosomal imbalance. The important factor is dosage compensation. Only one X in each cell needs to be fully active. Thus, potentially detrimental effects of an X chromosomal imbalance are mitigated (although not exactly canceled out) by *in*activating a supernumerary or abnormal X, or by *not* inactivating a sole remaining X, as the case may be.

The conceptus with an X chromosome complement in excess of the normal 46,XX or 46,XY accommodates to this imbalance by inactivating any additional X chromosome; or, as Migeon (2007) emphasizes, by maintaining, in each cell, just one X in the active state. This is nearly successful in the 47,XXX female and the 47,XXY male, in whom there is apparently normal in utero survival and a relatively mild postnatal phenotype (p. 478). The fact that some loci are not subject to inactivation, and may therefore function in the disomic (XXY), trisomic (XXX), or even quintasomic (49,XXXXX) states, is likely the predominant reason for the phenotypic abnormalities associated with these karyotypes.

In females with abnormal X chromosomes, the pattern of X-inactivation is usually nonrandom, particularly when the imbalance due to the abnormality is "large." In the 46,X,abn(X) karyotype, with one normal X and one abnormal X—an "abn(X)," as we write it here—the abnormal X is characteristically the inactive one. However, if the abnormality is a very small deletion or duplication, the inactivation pattern can be random. In the case of the X-autosome translocation heterozygote, the normal X is usually, although not invariably, inactive (Chapter 6).

Laboratory Test for X-Inactivation

Analyzing the pattern of X chromosome methylation with molecular methodology shows whether inactivation is random or nonrandom. A useful assay is methylation-specific polymerase chain reaction (PCR) based on the androgen receptor gene, located at Xq13 (or any other gene with a convenient polymorphism). A highly skewed pattern, with one X mostly methylated and the other mostly not, is indicative of nonrandom inactivation (Kubota et al., 1999). While this test is performed routinely on a blood sample, there are grounds for believing that the assay result may fairly represent the state in other body tissues (Bittel et al., 2008). The former tests of Barr body (Fig. **13–1**) and late-labeling BrdU analysis (Fig. **13–2**) are of historic interest; both assays provide a nice visual illustration of the concept of X-inactivation.

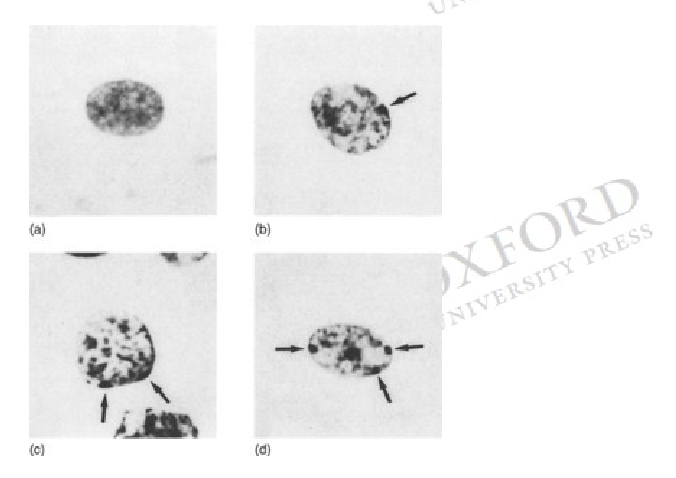




Figure 13–1

Buccal mucosal cells from (*a*) a 45,X female, with no Barr body present; (*b*) a 46,XX female showing the inactive X as a Barr body; (*c*) a 47,XXX female showing two Barr bodies; and (*d*) a 48,XXXX female with three Barr bodies.

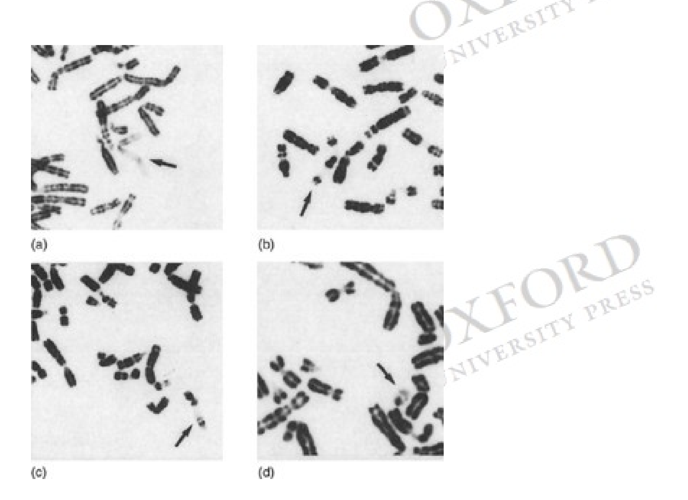




Figure 13–2

Partial metaphases showing X-inactivation: (*a*) a normal X chromosome, (*b*) an isochromosome of X long arm, (*c*) an X with a short arm deletion, and (*d*) a ring X. BrdU had been added for the last 6 hours of culturing. The inactive chromosomes, replicating at this late time in the cell cycle, incorporate BrdU extensively, and thus are palely stained. The active X stains darkly.

Details of meiotic behavior

Meiosis proceeds differently in each of the various sex chromosome abnormalities, and each warrants separate consideration.

XXX

On theoretical grounds, one might expect the three X chromosomes to display 2:1 segregation, with the production of equal numbers of X and XX ova. But this is not the case. No discernible increased risk for chromosomally abnormal offspring of these women has been demonstrated: in the extensive review of Otter et al. (2010), only one case had ever been reported of an XXX mother having had an XXX daughter. Apparently, only normal ova, with a single X, are regularly produced. It may be that the extra X is lost before meiosis occurs (Neri, 1984), with meiosis then proceeding as in the normal XX female. A few instances of premature ovarian failure (POF) in 47,XXX women are on record, including even in adolescence (Holland, 2001). But since XXX and POF are both fairly common, cause and effect remain uncertain.

XXY and xxy mosaic states

Barring medical intervention, infertility is almost inevitable in Klinefelter syndrome, although some remarkable exceptions exist. Terzoli et al. (1992), for example, report an XXY man who had fathered a daughter, with paternity testing confirming fatherhood, and they quote two other such cases. Undetected XY/XXY mosaicism could account for some of these cases. Bergère et al. (2002) showed both XY and XXY cell populations in testicular biopsies from three of four men who, on blood karyotyping and fluorescence in situ hybridization (FISH) analysis, were nonmosaic 47,XXY. These three men had small numbers of sperm identified in the biopsied tissue (one went on to have a child by in vitro fertilization, IVF). Several workers have karyotyped sperm from XXY men, and all find an excess, albeit not a large one, of 24,XX and 24,XY sperm. Possibly, these XY and XX sperm come from XXY spermatogonial stem cells. Alternatively, the abnormal gonadal environment may of itself predispose to gonosomal nondisjunction in the XY tissue, and from that stance autosomal segregation may also be vulnerable; consistent with this observation is a higher rate of disomy 21 on sperm studies (6.2% vs. 0.4% in controls) (Hennebicq et al., 2001; Bergère et al., 2002).

Medical intervention may allow men with Klinefelter syndrome to become fathers. This requires the procedure of testicular sperm extraction (TESE): the surgical opening of the testis, with microdissection of seminiferous tubules under the operating microscope, and analysis on site by an embryologist for the presence of sperm; the procedure is preferably undertaken on the day before programmed oocyte retrieval from the female partner. The few single sperm obtained are injected into the egg (intracytoplasmic sperm injection, ICSI).[1] The success rate is variable, with sperm retrieved in about 40%–70% of men, and about half of couples achieving pregnancy (Denschlag et al., 2004; Schiff et al., 2005). The chromosomal outcome for the child appears promising, with only one instance known of fetal XXY (and none of fetal XXX). In one triplet pregnancy, the three karyotypes at chorionic villus sampling were 46,XX, 46,XY, and 47,XXY; fetal reduction was done at 14 weeks, leaving XX and XY twins (Ron-El et al., 2000). However, on examination at a much earlier stage, in the embryo at preimplantation genetic diagnosis (PGD), the rate of aneuploidy, both for the sex chromosomes and autosomes 18 and 21 in particular, may be higher from XXY patients; and this is consonant with the observed higher rate of disomy 21 on sperm study mentioned earlier. Thus, PGD could be offered to these couples (Staessen et al., 2003).

As for mosaic states, Giltay et al. (2000) have studied men presenting with severe oligospermia, among whom there were cases of XY/XXY and XY/XXY/XXXY. Applying FISH to sperm analysis, and probing for chromosomes 18, X, and Y, the aneuploidy rate was somewhat increased compared to a normal population, although in fact similar to a group of normal 46,XY men with oligoasthenoteratospermia who were ICSI candidates. It may be that the sperm abnormalities reflect the testicular defect per se, rather than being a direct consequence of the XXY constitution. Studies in the XXY mouse support this interpretation (Mroz et al., 1999).

XYY

The clinical observation is that XYY men have no *discernible* increase in risk to have children with a sex chromosome aneuploidy (and XYY or XXY would have been the theoretical risks, from an XYY trivalent at meiosis). A true increased risk of a fraction of a percent could be distinguished only with the greatest difficulty when the background population risk is of a similar order of magnitude. On laboratory study, XYY spermatocytes proceeding through meiosis encounter checkpoints that lead to elimination of most of the abnormal forms (Milazzo et al., 2006),[2] but nevertheless, some men may have a small increased fraction of 24,YY and 24,XY spermatozoa in the ejaculate, and in some also, of autosomal disomies. A distinction may be drawn between XYY men presenting with infertility, and those whose fertility is intact, with the sperm aneuploidy rate somewhat higher in the former. Rodrigo et al. (2010) studied the next stage of development, namely, the preimplantation embryo, from five infertile XYY men having had IVF. The rates of chromosome abnormality were double that of a control group, with particular elevations in XY aneuploidy and triploidy.

45,X turner syndrome

The great majority of women with 45,X Turner syndrome (TS) are infertile and do not spontaneously menstruate or develop secondary sexual characteristics. The ovaries

initially appear to be normal but begin to degenerate in midfetal life. Oöcytes undergo apoptosis and disappear at an accelerated rate and, in most cases, are gone by the age of 2 years: “the menopause occurs before the menarche” (Federman, 1987; Modi et al., 2003). Spontaneous menstruation is uncommon but recorded (Lippe, 1991; Hovatta, 1999), and in one series of eighteen 45,X girls, none had ovarian follicles on biopsy in childhood or teenage (Borgström et al., 2009). Completed pregnancy in women with an apparent 45,X karyotype is very rare: in a Danish study based on a national TS register, none of 200 45,X women achieved a natural pregnancy (one had twins by ovum donation) (Birkebaek et al., 2002). Sybert (2004) records a total of 18 cases, these women having had 42 pregnancies, of which 17 proceeded to live birth, including one with trisomy 21, and two with 45,X.

What is the explanation for fertility in these cases? An obvious point to consider is gonadal mosaicism, with a 46,XX cell line in the ovary. This has often been suggested, but rarely proven (Birkebaek et al., 2002). Jacobs et al. (1997) undertook a systematic search in 84 subjects with TS whose standard blood karyotype was 45,X, with molecular testing of blood and of a second tissue (buccal cells) and found only two cases of X/XX mosaicism. One very thorough study is that reported in Magee et al. (1998b), concerning a 45,X woman who had had seven pregnancies, five miscarrying, one producing a healthy male, and the last terminated following demonstration of fetal cystic hygroma and a 45,X karyotype on amniocentesis. Biopsies of skin, uterus, and ovary at subsequent gynecological surgery all gave a 45,X karyotype, but molecular testing showed two alleles in ovarian DNA, indicating the presence of occult 46,XX tissue. A subtler consideration is whether a pure 45,X oöcyte could in fact proceed through meiosis I, given that the sex chromosome has no homolog with which to pair.

X/XX, X/XX/XXX, and X/XXX mosaicism turner syndrome

The relative fractions of the various karyotypes are listed in Table 13–1. For practical purposes, one should make a distinction between those mosaic women who display, to some extent, a TS phenotype, and in whom the fraction of 45,X cells is substantial, versus those of normal phenotype, who have only a low proportion (the latter noted separately later). The risk for chromosomally abnormal offspring hypothetically depends upon the degree and, crucially, distribution of the 45,X cell line. If the gonad contains 45,X cells—in other words, if there is somatic-gonadal mosaicism—and perhaps if these abnormal cells’ survival is enabled by support from surrounding 46,XX oögonia, a true increased risk may exist, although there is difficulty in assessing to what extent the ascertainment in published reports has been biased. Sybert (2005) lists 92 pregnancies to 34 X/XX TS mothers, with only 39 of these proceeding to live birth, and six of these babies with an X chromosome abnormality, and one with trisomy 21. In the Danish survey of Birkebaek et al. (2002), 27 out of 78 women with X/XX or X/XX/XXX mosaic states had had at least one child, of whom one was 45,X/46,XY with ambiguous genitalia. Uehara et al. (1999c) record the exceptional circumstance of a woman with 45,X/46,XX having had three monosomic X pregnancies, all showing fetal hydrops; she also had a normal son.

Table 13–1. Relative Frequencies of Turner Syndrome Karyotypes

STANDARD MONOSOMY	**45,X**	**46%**
X mosaicism	X/XX, X/XXX, X/XX/XXX	7%
Isochromosome Xq	45,X/46,X,i(Xq), 46,X,i(Xq)	18%
Ring	45,X/46,X,r(X)	16%
Deletion Xp	45,X/46,X,del(Xp), 46,X,del)Xp)	5%
Structural abnormality of Y		6%
Other		2%

Source: Jacobs et al. (1997).



The variability of phenotype according to the degree of mosaicism is well illustrated in the report of Lespinasse et al. (1998), who studied monozygous (but not identical) triplets with 45,X/46,XX mosaicism. One child with typical TS had only 6% 45,X cells on blood karyotyping but 99% of fibroblast analysis. One sister with only mild features to suggest TS had 43% of fibroblasts with 45,X, and the third sister, of normal phenotype, had just 3%. Presumably, the mosaicism existed from a very early stage, and the three-way division of the 45,X/46,XX blastocyst, or (if marginally later) of the inner cell mass, happened to cut across an asymmetric disposition of normal/monosomic cells.

Low-level 45,X/46,XX Mosaicism in Phenotypically Normal Women.

This category is to be distinguished from that of TS due to 45,X/ 46,XX mosaicism discussed earlier, and it is likely to be without reproductive consequence. Loss of one X (or one Y) to give an occasional 45,X cell is a normal characteristic of ageing in the 46,XX female (or 46,XY male). Nowinski et al. (1990) studied women who had presented for chromosome testing for a variety of reasons and found X chromosome loss (XCL) to be a consistent age-related phenomenon. Russell et al. (2007) reviewed data from a large number of females having had a peripheral blood cytogenetic analysis and correlated the degree of XCL with age, again documenting a clear association. Up to age 30, 1% or less of cells showed XCL, but rising to an average 2%, 3%, and 5%, at median ages of 40, 50, and 65 years, respectively. To give a sense of where a threshold might lie between normality and the possibility of a significant effect, we may consider the 99th centiles of the fractions of observed XCL, for different cell counts. On a 30-cell count, for example, the 99th centiles were as follows: 6% at 30 years, 9% at 40, 13% at 50, and 17% at 60. These workers also identified an absence of any XCL effect in relation to reproductive loss or infertility, in agreement with Nowinski et al. (although some other studies have contradicted this). Testing a different tissue, such as buccal mucosal cells by FISH, might seem attractive as a means to detect true constitutional X/XX mosaicism, although this may only be useful at higher levels of mosaicism (Schad et al., 1996).

X,abn(X) turner syndrome variants

Fertility may be retained in some TS variants resulting from an effective partial X monosomy. Deletion of Xp, deletion of Xq, isochromosome Xq, and large ring X are the major categories (Fig. **13–3**). In these cases, mosaicism with 45,X or 46,XX cell lines is common. Presumably a partial synapsis occurs at meiosis in the 46,X,abn(X) oöcytes, with the intact segment of the abnormal X pairing with the homologous region of the normal X. A 1:1 segregation would be expected, with equal frequencies of gametes carrying either the normal X or the abnormal X, from that part of the gonad tissue containing the abn(X).

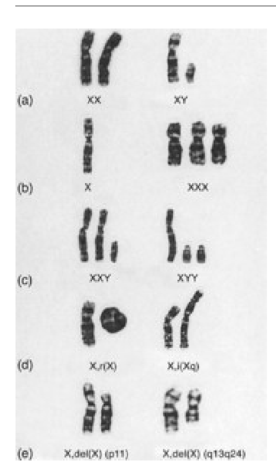






Figure 13–3
Some sex chromosome complements: (*a*) normal female XX and normal male XY; (*b*) X and XXX females; (*c*) XXY and XYY males; (*d* and *e*) abnormal chromosomes from females with a ring X, an isochromosome of X long arm, an X short arm deletion, and an X long arm deletion.

X deletions, pter or qter.

Simpson and Rajkovic (1999) summarized the recorded data at that time, with respect to terminal X chromosome deletions, relating the functional ovarian phenotypes, and their summary diagram is reproduced in Figure **13–4**. Lachlan et al. (2006) reviewed their own experience and the published literature, and they noted fertility with respect to these terminal deletions of the X: p11.4, p21, p21.1, p22.1, p22.12, and p22; and these interstitial deletions: p21.1p11.3 and p22.33p22.12. Transmission from a carrier mother is recorded, and the implications differ according to the gender of the child.

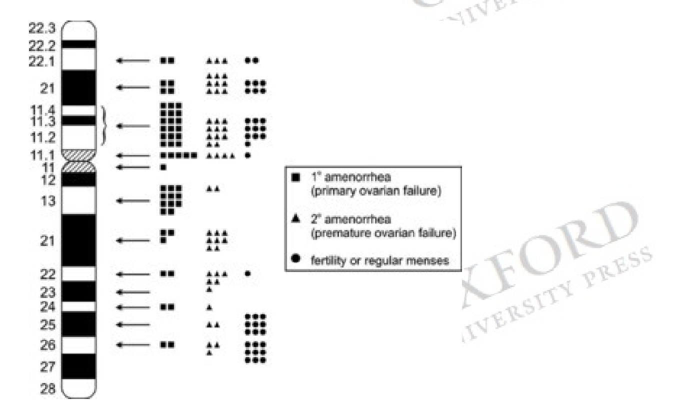




Figure 13–4
Terminal Xp and Xq deletions and the associated ovarian functional phenotype, according to position of the breakpoint. (From J. L. Simpson and A. Rajkovic, 1999, Ovarian differentiation and gonadal failure, *American Journal of Medical Genetics* 89:186–200. Courtesy J. L. Simpson; reproduced with the permission of Wiley-Liss.)

Mother-*daughter* transmission of a del(Xp), and of a del(Xq), are on record and indeed presumed four-generational matrilineal transmission (Wandstrat et al., 2000; Karaman et al., 2003). Palka et al. (1994) describe an apparently nonmosaic 45,X woman who had an abnormal child with an interstitial Xp deletion, del(X)(p22.2::p11.3). Upon restudy, the mother herself had one 46,X,del(X) out of 450 cells, allowing the presumption of a somatic-gonadal mosaicism. In a more direct demonstration of gonadal mosaicism, Varela et al. (1991) studied a woman with TS and normal menstruation and who had a 46,X,del(X)(p21) daughter. They showed 5/100 cells with 46,X,del(X)(p21) in one ovary, while all cells from the other ovary, fibroblasts, and lymphocytes were 45,X. Gonadal function can vary in a family, as Zinn et al. (1997) show for a familial del(X)(p21.2). The 45,X/46,X,del(X) mother had three pregnancies, including one miscarriage, and had normal menses till age 39. Her two daughters were both 46,X,del(X). The elder was amenorrheic at age 15, while the younger had spontaneous menarche at age 14½, with regular cycles 1 year later. A very similar family is on record in Adachi et al. (2000). An X deletion may be sufficiently unremarkable in its phenotypic effect in the female that it is not suspected in the mother, but only discovered fortuitously, such as at prenatal diagnosis (Wandstrat et al., (2000). It is to be noted that some X chromosomes with deletions may actually be combined del(Xp)/dup(Xq), or vice versa (Giglio et al., 2000).

Transmission to a *male* conception will almost always lead to nonviability, due to nullisomy for the segment concerned. In the case of terminal Xp, the limit of viability for the hemizygous deletion male is defined by the cases studied in Melichar et al. (2007), with the maximum survivable loss being ~10 Mb. The phenotype is very abnormal and includes severe psychomotor retardation, Léri-Weill syndrome, chondrodysplasia punctata, ichthyosis, Kallmann syndrome, and ocular albinism.

Ring X Chromosome.

Rare reports of fertility exist. Blumenthal and Allanson (1997) record a woman with mosaic ring X Turner syndrome, 45,X/ 46,X,r(X), who had been amenorrheic until being given hormone replacement therapy. She had three pregnancies: a healthy 46,XY son, a 12-week miscarriage, and a healthy daughter with the same 45,X/ 46,r(X) karyotype. The latter was presumably 46,X, r(X) at conception, with postzygotic loss of the r(X) in some tissue. Other such cases are known (Uehara et al., 1997). A rather different example is that in Matsuo et al. (2000), in which a mother and daughter were 45,X/46,X,r(X)(p22.3q28), the ratios of X:Xr(X) being 97:3 in the mother and 73:27 in the daughter. The ring comprised an almost complete X, but small distal Xp and Xq segments were deleted. The two X chromosomes were randomly inactivated, and in consequence, presumably, some "brain genes" would have been functionally nullisomic in those cells having the normal X-inactivated. Thus, mental function in the mother, and more so in the daughter, was compromised. A male with r(X) is almost unknown, but Ellison et al. (2002) describe transmission from a nonmosaic 46,X,r(X) mother to her nonmosaic 46,Y,r(X) son, mother and son both short statured. The breakpoints were very distal, within and beyond the Xp and Xq pseudoautosomal regions, respectively.

The "tiny ring X syndrome" due to absence of *XIST* with a functional X disomy is a quite different clinical entity, typically associated with severe mental retardation, and is discussed on p. 484. Some tiny *XIST*-lacking ring X chromosomes can yet be associated with only a Turner phenotype, likely reflecting different characteristics, or tissue distributions, of the abnormal chromosome (Turner et al., 2000).

45,X/46,XY and 45,X/47,XYY mosaicism in the male

X/XY mosaicism is occasionally found in males presenting with hypogonadism and infertility with oligo/azoöspermia; in some, the Y chromosome has a deletion at Yq11 (Telvi et al., 1999; Cui et al., 2007). The maleness presumably reflects the fact that the gonad contained XY cells with a functioning *SRY* gene, that were able to induce effective testicular differentiation with consequent androgenizing capacity. Reddy and Sulcova (1998a) did testicular biopsy on an X/XY man and demonstrated absence of spermatogenesis; about half of the Sertoli supporting cells showed a Y-signal on FISH. One X[10]/XY[90] man with moderate oligoasthenoteratozoöspermia showed a two- to three-fold rate for XY disomy and 18 disomy in sperm (using 18 as a representative autosome) (Newberg et al., 1998). In contrast, a man with 45,X/47,XYY mosaicism reported in Dale et al. (2002) showed normal gonosomal complements in 99.9% of sperm. He had presented with infertility due to oligospermia; a normal 46,XY pregnancy was achieved with ICSI.

X microdeletions

We confine our discussion to deletions for the demonstration of which molecular cytogenetic and array comparative genome hybridization (CGH) methodology is applied. The risk to transmit the abnormal chromosome will presumably reflect equal segregation, 1:1. If passed from a 46,X,del(X) mother to a *daughter*, the daughter's phenotype may be the same as that of the mother (which may well be quite normal). But a firm statement cannot be made. For example, Grillo et al. (2010) identified a novel 1.1 Mb deletion at Xq22.1 in the severely retarded daughter of a mildly retarded mother; X-inactivation skewing, and an influence of the paternal genotype, may have been the basis of the differences in phenotype. If passed from a 46,X,del(X) mother to a 46,Y,del(X) *male* conceptus, the hemizygous male fetuses will be nullisomic for loci within the region of the deletion. Viability may be possible, but the absence of loci will lead to a “contiguous gene syndrome.” A classic example is the variable combination of Duchenne muscular dystrophy, retinitis pigmentosa, adrenal hypoplasia, glycerol kinase deficiency, and mental retardation, due to microdeletion within Xp21 (Worley et al., 1995). Numerous “neurocognitive loci” are located in proximal Xp, and Qiao et al. (2008) implicate loss of such loci as the cause of autism and intellectual disability in two brothers, due to an Xp11.22 deletion inherited from their mother; a craniofacial phenotype may also accompany this deletion.

The carrier female may also display, at least to some extent, the components of a contiguous gene deletion. We have seen a young woman with chronic granulomatous disease, retinitis pigmentosa, and ornithine transcarbamylase (OTC) deficiency, having an Xp deletion shown on FISH, these loci being within about 1 Mb at Xp11.4p21.1. She suffered recurrent upper respiratory tract infections and had a history of surgery for mastoid osteomyelitis and lung abscess; her peripheral vision was poor; and she unconsciously self-managed the OTC deficiency by avoiding high-protein foods. X-inactivation was random, on blood analysis. She came to prenatal diagnosis and elected to terminate a male pregnancy with the deletion, the predicted phenotype being severe (Coman et al., 2010b).

X duplications

In the rare case of the abnormal X having a duplication of X material, 1:1 segregation in the female heterozygote would be expected. Offspring inheriting the abn(X) are hemizygous males or heterozygous females. Duplications may be “classical,” and detectable cytogenetically, or “microduplications,” requiring molecular methodology for their recognition. The basis of the rearrangement can be a direct duplication, an inverted duplication, or an isodicentric chromosome (James et al., 1997; Shapira et al., 1997a; Matsuo et al., 1999; Kokalj Vokac et al., 2002). The typical karyotype is 46,X,dup(X), although mosaic forms are on record, such as 46,XX/46,X,dup(X) and 45,X/46,X,iso dic(Xq).

Hemizygous sons, in whom the abn(X) is genetically active, have a functional partial X disomy, and are of abnormal phenotype, often severely so. Two notable long arm duplications are Xq21.1–q21.31 and Xq27.2–qter, both leading to a clinical phenotype resembling Prader-Willi syndrome, and which can be transmitted from a carrier mother, who may or may not be phenotypically normal herself (Sanlaville et al., 2009). Gabbett et al. (2008) describe a boy with poor motor and language development, who became a “food seeker” as an infant, and in whom a dup(X)(q21.1–q21.31) was identified. His mother, who had had “learning difficulties” at school, proved (on blood analysis) to be a mosaic carrier of the duplication, with a random pattern of X-inactivation.

The phenotype in *heterozygous daughters* is less predictable. If the rule of selective Lyonization holds, the abn(X) is consistently the inactivated one, and normality might, in theory, be expected; while if the rule fails, random inactivation could, in theory, lead to an attenuated functional partial disomy, with phenotypic abnormality. Thus, Apacik et al. (1996) report a normal grandmother and a mother with a duplication of Xq12-q13.3, due to an inverted insertion. In them, the abn(X) was preferentially inactivated. The family only came to attention when the mother had two retarded and dysmorphic sons with 46,Y,dup(X). Tzschach et al. (2008) report a similar circumstance in families segregating a dup(X)(p11.3p21.1) and a dup(X)(p22.11p22.2): the males severely retarded, the females all healthy. Numerous examples exist of random X-inactivation associated with abnormality (Matsuo et al., 1999; Monnot et al., 2008). Armstrong et al. (2003) describe an abnormal child with 46,X,dup(X)(q22.3q26) in whom the dup(X) was preferentially inactivated, but with a part of the duplicated segment apparently escaping inactivation. They propose functional disomy restricted to this small part to have been the cause of the observed anomalies. Somewhat similarly, Kokalj Vokac et al. (2002) showed consistent inactivation of the dup(X) in a girl with a de novo 46,X,dup(X)(p11.23-p22.33::p11.23-p22.33), except for the intriguing observation of early-replication within the actual breakpoint region; this may have been the basis of her abnormal phenotype. But theory quite often does not apply in practice, and instances are recorded of random X-inactivation with associated normality, and preferential inactivation in the setting of phenotypic abnormality (Matsuo et al., 1999). Stankiewicz et al. (2005) report a dup(X)(q26.2q27.1), a 7.5 Mb segment, passed from mother to daughter, and both phenotypically abnormal, in spite of skewed inactivation. Interstitial microduplication of the region on Xq28, which includes the *MECP2* gene (Lubs syndrome), is noted on p. 332.

A recurrent microduplication, which includes, but is not necessarily confined to, the segment Xp11.22-p11.23, has the curious quality that in most affected females, it is the *normal* X that is preferentially inactivated (Holden et al., 2010). Mental retardation, borderline to severe in nature, may affect males and females similarly. The phenotype is notable for including a particular abnormal electroencephalographic (EEG) pattern, in the absence of obvious seizures. The inheritance could be described, in most, as X-linked dominant; for the smallest duplication, transmission follows an X-recessive pattern.

Sex chromosome polysomy

The 48,XXXX female characteristically has diminished ovarian function, and fertility in pure XXXX is on record in only one case (ascertained through a Down syndrome child) (Gardner et al., 1973b). Sterility is presumably invariable in XXXY and XXYY males, who have a further sex chromosome superadded upon the Klinefelter karyotype (Linden et al., 1995).

Y chromosome abnormality

Y chromosome deletions associated with male infertility are discussed in Chapter 23. A single family is recorded with an interarm insertional Yq duplication, presumed transmitted from a normal father to two normal sons. The wife of one had presented with two miscarriages, which may or may not have been related (Engelen et al., 2003).

Genetic counseling

Many of the gonosomal disorders are associated with infertility, or at least subfertility. Some present a phenotype of relatively mild abnormality. Whether prenatal diagnosis is chosen, in those who are able to achieve pregnancy, may depend on the parents’ perception of the seriousness of the potential abnormal outcome. Their decision may well also be influenced by how difficult it was to achieve the pregnancy. Inference from prenatal X-inactivation analysis, in abn(X) cases, may be fraught with uncertainty.

XXX

XXX mothers have no discernibly increased risk of bearing chromosomally abnormal children. A theoretical increased risk for children with an X aneuploidy has not been demonstrated in practice. Despite reports of chromosomally abnormal children born to XXX women, it should be emphasized, as did Dewhurst and Neri in 1978 and 1984, respectively, that when biased ascertainment is taken into account, no excess of abnormal offspring has been reported. Near-silence subsequently in the literature on this issue suggests at least a rarity of abnormal pregnancy outcomes; one such case, an XXX daughter of an XXX mother, is mentioned in passing in Haverty et al. (2004). An additional risk estimate of <½% for a chromosomally abnormal child may be reasonable. A possibility of premature ovarian failure with 47,XXX can be brought to the attention of these women, which may assist in decisions about the timing of childbearing.

XXY

Hardly ever will these men father children, without recourse to IVF (see earlier). The early data are certainly small (Fullerton et al., 2010), but one may propose an approximate risk figure of 2% for a sex chromosomal abnormality in the child. Sperm and PGD chromosome studies indicate that as well as this small increased risk of gonosomal aneuploidy, autosomal aneuploidy might also be implicated, albeit that an actual case in a child is yet to be observed (Staessen et al., 2003). In younger men in whom the diagnosis of XXY is made, and who do have sperm in the ejaculate, gamete banking may be appropriate (Schiff et al., 2005; Ichioka et al., 2006). Indeed, given a greater success rate in younger men, Ferhi et al. (2009) advise that the procedure is best undertaken before the age of 32 years, which they see as a critical cutoff. A concomitant AZF deletion (p. 391) may warrant checking, and particularly in mosaic men with azoöspermia (Mitra et al., 2006).

XYY

To our knowledge, there is no report of a discernibly increased risk for the XYY male to have chromosomally abnormal children. A slight increase in gonosomal imbalances in sperm (see earlier) might nevertheless lead some to choose prenatal diagnosis. The risk might be greater in those XYY men who need fertility treatment (Wong et al., 2008), and for whom PGD might therefore be appropriate.

45,X Turner Syndrome

Natural fertility is very rare. However, a 45,X woman who has spontaneous menses may possibly be fertile. Endocrine and ultrasound studies may clarify whether ovulation is occurring, or likely to occur (Mazzanti et al., 1997; Paoloni-Giacobino et al., 2000a). Any period of fertility is likely to be shortlived; thus, a woman with 45,X TS who wishes to have a child should not delay in trying for a pregnancy.

Tarani et al. (1998) reviewed the literature on pregnancy outcome in (apparently) nonmosaic TS women. In all, fifteen 45,X women had 26 recorded pregnancies, with 9 miscarriages and 16 completed pregnancies. From these 16 pregnancies there were 13 normal children (81%), 2 stillborn, and 1 with Down syndrome. In further data from Sybert (2005), pregnancies with 45,X and 46,X,del(X) are listed. While the miscarriage rate is high, it may be that the maternal gynecology was more contributory than were fetal factors. There may have been selective reporting in the literature of those with abnormality (although it is true that any natural pregnancy in a 45,X woman might warrant publication), but nevertheless the conclusion of an increased risk for a chromosomal abnormality seems inescapable.

For the great majority of TS patients who cannot make their own eggs, ovum donation with IVF may be one route to achieve childbearing (Hovatta, 1999). Foudila et al. (1999) report their experience with 18 women with TS, and although the rates of embryo transfer were similar to those of other women with primary ovarian failure, the miscarriage rate was high (40%); possibly, this may have been due to uterine factors. Bodri et al. (2009) report a similarly discouraging experience. Fénichel and Letur (2008) insist on the advisability of transferring a single embryo only. Any genetic risk to the TS patient bearing children via ovum donation is due to that of the biological donor parents. A related donor (mother, sister) would have obvious attraction, and the improving methodology of ovum storage offers the possibility of maternal donation well ahead of the time of potential use (Schoolcraft et al., 2009). Gidoni et al. (2008) report a 33-year-old mother having “oöcyte vitrification” for the potential use of her daughter, with isoXq Turner syndrome. These authors discuss the ethical issues involved and conclude that the procedure is reasonable and acceptable, with the mother's motives purely altruistic, and she “is simply providing an option for her daughter.” Anticipating possible artificial fertility, the offer should be made of hormone treatment from the age of 10–12 years, in order to avoid uterine hypoplasia (Leclercq et al., 1992). Otherwise, obstetric management must take account of possible cardiovascular complication (Fénichel and Letur, 2008).

Mosaic 45,X turner syndrome

Women with 45,X mosaicism *and* a TS phenotype presumably carry the 45,X cell line in much of the soma and gonad. Categories include X/XX, X/XXX, and X/XX/XXX. Ovarian function is often intact, although premature failure is common (Blair et al., 2001; Sybert, 2005). The risk for miscarriage is increased (Homer et al., 2010). There is apparently an increased risk for X monosomy in a child, and this is consonant with theoretical expectation (Sybert, 2005). Normal cells in the gonad may provide support for monosomic cells that otherwise would not have survived. The upper limit of the risk may be about 15% (Tarani et al., 1998). In those having suffered ovarian failure, ovum donation may succeed.[3] A few may, as adolescents, have suitable follicles available for biopsy and thus be candidates for attempted ovum vitrification for possible future use (Borgström et al., 2009; Lau et al., 2009).

Low-Level 45,X/46,XX Mosaicism in Phenotypically Normal Women

This category is, for practical purposes, to be distinguished from that of the preceding section on “Mosaic 45,X Turner Syndrome.” The discovery of a low-level (a single-digit percentage) of 45,X cells in a woman presenting no phenotype traits of TS is not to be overinterpreted, nor is a reproductive risk to be exaggerated. Indeed, no such risk may apply (Horsman et al., 1987). Loss of an X chromosome is a normal concomitant of ageing (see also “Biology” section).

X,abn(X) turner syndrome variant

Not infrequently, women with incomplete Turner phenotypes due to a 46,X,abn(X) karyotype have normal secondary sexual development, and fertility is likely or indeed proven. The majority involve deletions of Xp or Xq, and ring X chromosomes. The deletions may be of quite substantial size, with a phenotypic range from partial TS through minor menstrual abnormality. Premature ovarian failure is likely, and it is practical advice that childbearing should be embarked upon earlier rather than later. Assuming 1:1 segregation (a fair assumption), the deleted X will be transmitted in 50% of ova. In Tarani et al.'s (1998) review, examples are given of TS mothers with Xp deletions and ring X chromosomes, most of whose children (5 out of 6) were abnormal, with the same type of TS. It might be guessed that this weighting toward abnormality was due to publication bias.

If the ovum containing a deleted X meets an X-bearing sperm, a conceptus with the same karyotype as the mother results. If the ovum meets a Y-bearing sperm, a zygote with partial nullisomy X results, and it may end in abortion, depending on the size of the deletion. Viable offspring are in the ratio of 1:1:<1 of chromosomally normal males, normal females, and X,abn(X) females. Mosaicism in the mother may well be reflected in mosaicism in the daughter. Uehara et al. (1997) record a 45,X/46,X,r(X) mother to whose child she transmitted the ring chromosome; the ring was present in 4% of the mother's cells and in 34% of the daughter's. A similar case is reported in Blumenthal and Allanson (1997), in this instance the mother having been on hormone replacement therapy.

X/XY male

Infertility is probable. If there is any sperm production, an IVF pregnancy might be possible. Given a possible increased risk for aneuploidy, gonosomal or autosomal (Newberg et al., 1998), PGD is to be considered in that setting.

X microdeletion

The carrier can be fertile, and 1:1 segregation with respect to the normal X and the abn(X) is to be expected. This implies a 50% risk to have a son affected with the full "contiguous gene syndrome" or a daughter who might display a partial phenotype. There may be an increased fetal loss rate with the 46,Y,del(X) karyotype. Prenatal diagnosis, if chosen, should be a molecular genetic exercise. Measurement of X-inactivation would not offer a clear interpretation of risk adjustment, in the case of transmission of the abn(X). Preimplantation genetic diagnosis may be appropriate.

X duplication

The female carrier has a chance of 50% to transmit the normal X, and a risk of 50% to transmit the dup(X). If an abn(X) pregnancy proceeds to live birth, a son would be abnormal due to X disomy. A daughter is not necessarily protected by selective inactivation, and thus phenotypic abnormality is possible.

Sex chromosome polysomy

Many XXXX women are of low-normal or borderline intelligence, and the questions of fertility and genetic risk may well be raised by their carers. In fact, it appears that sterility is usual. XXXY and XXYY men are undoubtedly sterile.

Notes:

[1] It is an intriguing thought that, in those cases proceeding to fatherhood through intervention with assisted reproductive technology, the situation may be presented of the homogametic sex being the one to provide the greater quantum of gametes, albeit by a small margin: an extraordinary contrast from the typical vast imbalance due to the heterogametic male of the species.

[2] One theory has it that escape from normal meiotic inactivation of Y-borne genes, due to a Y bivalent having formed and thus not able to accompany the X into the sex vesicle, could be the cause of spermatogenic arrest (Turner, 2007).

[3] Ovum donation might be too successful. Makrakis et al. (2009) report a young woman, X/XX, who had three embryos from donated ova transferred, with all three implanting, and one dividing to give a monozygous pair: a quadruplet pregnancy. She underwent fetal reduction, and eventually she gave birth to twins.



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Parental Autosomal Aneuploidy

Chapter: Parental Autosomal Aneuploidy
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A QUESTION OF REPRODUCTION is usually an academic matter in individuals with a cytogenetically detectable functional autosomal aneuploidy. But toward the milder end of the phenotypic range, social and emotional development may be such that forming a stable relationship is possible. Some who lack that degree of maturity may yet have a social freedom that opens the possibility of a sexual encounter. Either on their own behalf, or through the agency of parents or other carers (whose agenda may include sterilization), such people may present to the genetic clinic. Ethical issues raised in this context are aired in Chapter 1. With some of the deletions and duplications of lesser genetic imbalance revealed by molecular techniques, the phenotypic effects may be very mild, with social and intellectual functioning well within the normal range.

The mosaic state may on occasion be associated with an apparently normal phenotype, or at least very close to normal. Some of these persons will have been identified by their having had a child with the imbalance for which they themselves have a low-level mosaicism. Some present with infertility. The theoretical risk will depend upon the extent to which the gonad carries the aneuploid line.

In contrast, some structural imbalances are without discernible phenotypic effect. Deletion or duplication of a small segment of euchromatin, or the presence of a supernumerary marker chromosome (SMC), is occasionally recognized fortuitously in normal and fertile individuals.

Biology

Parental Trisomy

Parental Trisomy 8 Mosaicism.

Mosaic trisomy 8 arises postzygotically, from an initially normal conceptus (Robinson et al., 1999). Habecker-Green et al. (1998) review reports of reproductive status in 46/47,+8 individuals, and there is only a tiny number of cases, usually in persons in whom the diagnosis would not have been suspected clinically. They describe a woman with mosaic trisomy 8 having a history of four spontaneous losses, including a 46,XX fetal death at 27 weeks; her next pregnancy produced an apparently normal 46,XX daughter. Rauen et al. (2003) report a woman who presented a more typical clinical picture of trisomy 8 mosaicism having a 46,XX child (phenotypic abnormality in the child probably reflected paternal characteristics). Mercier and Bresson (1997) studied an otherwise healthy man, whose partner's recurrent miscarriage was the presenting problem, and in whom the peripheral blood karyotype was 46,XY[92]/47,XY,+8[8]. On fluorescence in situ hybridization (FISH) analysis of 25,000 spermatozoa, 398 (1.6%) showed disomy 8, which compared with a rate in control sperm of 0.2%. It is perhaps surprising that such a low level of disomic 8 sperm should be associated with a high miscarriage rate (always assuming that the link is causal and not coincidental). We have seen a somewhat similar case, a man of above-average intelligence and excellent physical health, with infertility due to oligospermia, in whom low-level trisomy 8 mosaicism was shown on two separate blood samplings; in his case, one could not exclude that the abnormal cell line was confined to hematological tissue, and the oligospermia coincidental.

Parental Trisomy 18 Mosaicism.

This is extremely rarely recorded in adulthood, and Tucker et al. (2007) review in detail the range of phenotypes. Some had presented with a history of miscarriage, and some due to having had a child with trisomy 18. Because of the usual high rate of lethality of trisomy 18 in utero, the genetic risks obtaining in such persons would apply substantially to miscarriage. The risk will relate to the gonadal load of trisomic cells; this is not usually known, but some gametic studies are recorded. Bettio et al. (2003) report a woman of normal intelligence with 70% trisomic 18 cells on blood but none on fibroblast karyotyping, presenting with infertility. Ovarian biopsy showed 90% trisomic cells from right ovarian biopsies and a normal karyotype in left ovarian tissue. A man of normal intelligence and appearance, presenting with severe oligospermia, had approximately 50% trisomy 18 mosaicism on blood and buccal mucosal cell analysis, although only 3% in skin fibroblasts: on sperm study, there was a 10-fold increase in disomy 18, compared with control data, although the absolute fraction was small (0.68%) (Perrin et al., 2009). Both testes may be free of the trisomic line, as apparently in the father of a normal daughter described in Lim and Su (1998). He was of normal intelligence and worked as a sales representative, and had “slightly unusual facial features.” The trisomic line was found only in blood (76%) and not in skin fibroblasts, and the disomic 18 rate in sperm was similar to that of a control.

Maternal Trisomy 21.

At female meiosis, the classical scenario is that the three homologs form either a bivalent and a univalent, or a trivalent (Fig. **14–1**) (Wallace and Hultén, 1983). If the former, the bivalent may disjoin and segregate symmetrically, but the univalent passes at random to either daughter cell (1:1 +1 segregation). If the latter, a trivalent may of itself set the stage for aberrant segregation (2:1 segregation). In either case, the result is disomic (24,+21) and normal (23,N) gametes in equal proportions. Speed (1984) has observed trivalents in about 40% of meiotic cells and a bivalent plus a univalent in the remaining 60%. An alternative scenario is that the “third” chromosome 21 separates prematurely

into chromatids, and each chromatid then passes to a daughter cell (the oöcyte, and the first polar body). Cozzi et al. (1999) provide direct evidence for this mechanism in the FISH study of unfertilized oöcytes from a woman who was presumed to be a 46/47,+21 gonadal mosaic.

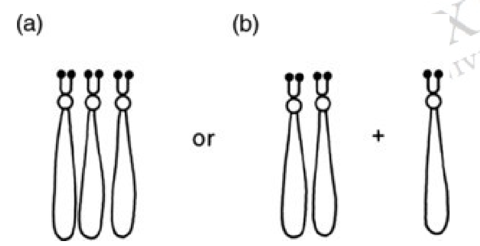


Figure 14–1
Possible synapsis of three no. 21 chromosomes: (*a*) as a trivalent and (*b*) as a bivalent and a univalent.

In a review of the literature, Shobha Rani et al. (1990) list 30 reports of pregnancy in Down syndrome (DS) women. The ratio of DS to normal offspring was 10:17 (there were three abortions), not significantly different from a 1:1 ratio, but suggestive of a deficit in trisomic offspring. A reasonable interpretation is that 46,N and 47,+21 conceptions occur with equal frequency, but loss of pregnancy is greater with the trisomic fetuses. About one-third of the 46,N offspring were nevertheless abnormal, which may have reflected paternal or environmental factors. Cunniff et al. (1991) noted a diminution in the number of oöcytes in the ovaries of DS girls at the time of birth, which could be the cause subsequently of subfertility.

Paternal Trisomy 21.

Spermatogenesis is reduced in the male with DS, but it does not necessarily fail; and a tiny number of examples of proven or suspected fatherhood in DS males have been documented (Pradhan et al., 2006).

Parental Trisomy 21 Mosaicism.

In practice, it is usually only those recognized mosaic individuals with a low percentage of +21 cells who seek genetic advice. These people typically come to notice because they are studied as apparently normal parents of more than one DS child (and see p. [link]). The important factor, if it could only be known, is the degree to which the gonad comprises 46,N and 47,+21 cells. The trisomic cells (see earlier discussion) produce disomic and normal gametes in equal proportion; of course, normal cells, other things being equal, give rise only to normal gametes. Thus, the proportion of abnormal gametes produced depends on the proportion of germ cells that are trisomic. In the limit, the gonad might be fully 47,+21. Any level of correlation between the degree of mosaicism in lymphocytes and gametes is not readily amenable to study. Familial trisomy 21 mosaicism is on record but is exceptional (Kovaleva, 2010).

Parental Partial Aneuploidy (46,rea)

Uncommonly, the unbalanced and phenotypically abnormal carrier of a classical cytogenetically detectable chromosomal rearrangement may be functionally fertile. The usual forms are deletion, duplication, and the derivative chromosome from an unbalanced translocation. Barber (2005) lists the recorded deletions and duplications that have been transmitted by partially aneuploid parents, with all but two of the autosomes represented at least once, and chromosomes 4, 5, 8, and 18 the most often seen (Table 14–1). Microarray comparative genomic hybridization (CGH) methodology is revealing many more examples of imbalance in which, due to the lesser extents of the loss or gain, a phenotype of lesser severity may more frequently allow the possibility of the heterozygote passing on the abnormality. While the rules of mendelian segregation impose a straightforward 1:1 ratio of normal:abnormal chromosome transmission, there is the potential complicating factor of variable expressivity, and indeed nonpenetrance, which might in some cases be due to the presence or absence of other imbalances elsewhere in the genome (Veltman and Brunner, 2010), and which we discuss also in Chapter 17. Some microarray-level imbalances are achieving syndromic status, such as del(3)(q29), which is noted in Chapter 19 (p. 312), but which could also have a rightful place, as a parental autosomal imbalance, in this chapter.

Table 14–1. Euchromatic Deletions or Duplications That Are on Record in the Literature as Having Been Transmitted from Parent to Child, and Having an Associated (and Presumably Causally Associated) Abnormal Phenotype

REGION	ASCERTAINMENT	REGION	ASCERTAINMENT
deletion:		*duplication*:	
1q42.1q42.3	Developmental delay, ADD	1q23q25	Mild MR and dysmorphism
2p11.2p12	Wilms tumor, developmental delay	2q24.3	Epilepsy
3p25pter	Speech delay	3q25.3q26.2	Microcephaly, congenital heart disease, and deafness
4p15.2p16.1	MR, dysmorphism	4q31.22q33	Mild MR and dysmorphism
4q33qter	Multiple congenital abnormalities and language delay	4q31.1q32.3	Developmental delay, nasal speech
4q33q35.1	Developmental delay	5q15q22.1	Hyperactive, mild MR
4q33q33	Developmental delay and dysmorphic features	7p12.2p13	Failure to thrive
4q32q33	Developmental delay and dysmorphic features	7p12.1p13	Short stature, ?Silver-Russell
5p15.32pter	Developmental motor, speech delay	7q32q36.1	Developmental delay, behavioral problems
5p15.31pter	Speech delay, dysmorphic	8p23.1p23.1	Congenital heart disease
5p15.3pter	Cat cry at birth, low birth weight	8p23.1p23.1	Developmental delay

5p15.3pter	Speech delay, hearing loss, mild MR	8p23.1p23.1	Developmental delay, hypotonia
5p15.3pter	Speech delay, mild developmental delay	8p22p23.1	Mild MR only
5p15.3pter	Speech delay, raspy voice	8p21.3p23.1	Congenital heart disease
5p15.3pter	Speech and developmental delay	8p21.3p23.1	Speech delay
5p15.1pter	Multiple congenital abnormalities	8p21.3p22 (or p22p23.1)	MR, short stature, hypertelorism
5p14p15.3	Cri du chat	8p12p21.1	Developmental delay
5p13.3p14.3	Microcephaly, small	9p22p24	Short, low IQ, dysmorphic
5p13.1p14.2	Speech delay	10p13p15	Developmental delay, especially speech
5p13p15.1	Maternal age	11q13.5q21 (or q21q23.1)	Maternal age
7q11.23	Williams syndrome	14q13q22	Developmental delay
8p23.1pter	Mental slowness, seizures	15q11.2q13	Developmental delay, hypogonadism
9q31.2q32	Developmental and growth delay, unusual appearance	15q11.2q13	Severe MR
11q24.2qter	Developmental delay	15q11.2q13	Developmental delay
13q14.1q21.3	Leukocoria	15q11q13	Developmental delay
14q31q31	Developmental delay	16q11.2q12.1	Speech delay
15q11q12	MR	18 cen-pter	Dysmorphic, moderate MR
18p11.3pter	Previous son with MR	22q11.2	Variable (p. 327)
18p11.21pter	MR, short stature	21q22qter	Unusual appearance
deletion:			
18p11.2pter	Abnormal ultrasound		
18p11.23pter	Multiple congenital abnormalities		
18p11.2pter	MR, short stature		
18p (pre-banding)	Failure to thrive, ptosis		
18q23qter	Dysmorphic		
18?q21qter	Multiple congenital abnormalities		
18q22.3qter	Phenotypic abnormality		
20p11.2p12.2	Dysmorphic		
21q11q21.3	Dislocated hips		
22q11.2q11.2	Cardiac failure		

ADD, attention-deficit disorder; MR, mental retardation; ?, suspected diagnosis.

Source: From the review of Barber (2005), with the addition of del 7q11.23, dup 2q24.3, and dup 22q11.2.

It is often observed that the child is more severely affected than the parent. In part, this will be due to bias of ascertainment: parenthood is more probable in those whose abnormality yet allows them to have acquired a degree of social functioning, whereas a more markedly affected child will likely come to medical attention, and only then is the parental state recognized. Another consideration may be the "genetic quality" of the other parent, and bearing in mind the strong tendency toward "positive assortative mating" with respect to intelligence. (Of course, any diminished "genetic quality" need not apply, if the other parent were intellectually disabled due to environmental factors, such as birth injury.)

Classical Deletions

Rarely, the parental phenotype may be severe, such as the cri du chat syndrome documented in mother and child in Martínez et al. (1993; and see p. [link]). Loss of a tumor suppressor gene, the *APC* gene which is the basis of familial adenomatous polyposis, accompanied a del(5)(q22q23.2) in a retarded man, his aunt, and inferentially his retarded mother, causing polyposis in at least the man and his aunt; other family members carried a balanced insertional rearrangement (Cross et al., 1992). A less severe clinical picture is more often observed, as may be exemplified by deletion for the segment 8p23.1-pter in Pettenati et al. (1992); a family photograph shows unremarkable physical appearances, but the del(8p) children had learning and behavioral difficulties. The well-known chromosome 22q11 deletion can be transmitted from parent to child, with a different clinical picture in each (p. 327); typically the child is more severely affected than the parent. Another well-recorded abnormality, the 18p deletion, has been reported due to familial transmission in six families (Maranda et al., 2006); and similarly, a number of instances are known of the parental transmission of the 7q11.23 deletion of Williams syndrome (Metcalfe et al., 2005). As a rather obvious rule, the smaller the deletion, the milder the phenotype: Sanford Hanna et al. (2001) describe a very small distal 1q deletion, del(1)(q42.1q42.3), in a mother and son with rather minor physical anomalies, the mother requiring “assisted living services,” and the son attending special education classes at the age of 13, working at approximately a third-grade level.

Classical Duplications

Transmitted duplications are slightly less often reported than are deletions. This lower frequency may represent an ascertainment bias based on microscopy because only large duplications that involve more than one band can be easily visualized. Those duplications that result in a slightly broader band are less likely to be seen, as compared to deletions that remove an entire band or a substantial part of a band. Based on array-CGH, duplications less than 500 kb are commonly observed, and they are usually inherited from a carrier parent (see Chapter 17).

Three-generation transmission of a dup(7)(p11.2p12) is recorded by Leach et al. (2007) in three affected family members having a “mild cognitive deficiency.” Glass et al. (1998) report a mother and daughter with a dup(2)(q11.2-q21.1), due to an (8;2) insertion, leading to a clinical picture of minor facial dysmorphism, short stature, mild intellectual deficit, and psychiatric disease (schizoaffective disorder in the mother, paranoid psychotic state in the daughter). With an insertion such as this, the genotype is presumably that of a pure 2q partial trisomy. An example of the parent being less affected than the child is given in Pazooki et al. (2007), the mother having a milder dysmorphism than her mentally retarded daughter, and both 46,XX,dup(6)(q21q22.1). We have seen a family with, inferentially, four-generation transmission of a duplicated chromosome (segment 10p14). One great-grandparent, we may guess, was a gonadal mosaic, and then in two separate branches of the family the duplication was passed down, causing considerable psychological and social morbidity as it did so (Voullaire et al., 2000a). (Only when the cytogeneticist gathered three families with the same dup(10p) for a study was it realized that they were, in fact, related.) Somewhat similar is the family in Arens et al. (2004), a four-generation family, but in this case there were some individuals with a balanced 3;5 translocation, three of whose offspring with an unbalanced form, producing a duplication of 5q22.1–5q31.3, went on to transmit the same imbalance to their children.

Genomic imprinting was an important factor influencing the effect of a familial ins(2;6)(p22.2; q22.33q23.3) which had been transmitted through three generations (Temple et al., 1996), discussed also on p. 359. A child with transient neonatal diabetes had a duplication for 6q22-q33 due to the insertion, which she had inherited from her father. He and his mother also had the same unbalanced karyotype, but neither had any history to suggest neonatal diabetes. It was the imprinting effect due to paternal transmission that resulted in the child's medical condition.

dup(15)(q11.2q13).

Proximal 15q is a vexing region, because of the need to distinguish between harmless euchromatic variants (see p. [link]) and a pathogenic dup(15)(q11.2q13). The distinction is to be made upon the absence or presence, respectively, of the Prader-Willi/Angelman critical region (PWACR). There is the added complication that this region of chromosome 15 is subject to imprinting, with phenotypic abnormality, including autism spectrum disorder, typically occurring in the setting of transmission from the mother; a milder phenotype extending into the normal range may apply to paternal transmission (Bolton et al., 2001) (see also p. 329).

> A complex story is that of the family in Gurrieri et al. (1999). A child with pervasive developmental disorder, atypical autism, and epilepsy had, on molecular analysis, a dup(15)(q11.2q12) of ~2 Mb size; his mother had a duplication in the same region, but not so extensive (~1 Mb), and the grandparents had normal chromosomes. Thus, the first duplication had arisen on transmission from the normal grandfather, and then on transmission from the normal mother to the affected child, the duplication expanded. The child's abnormality is likely due to aberrant expression of imprinted genes.

Microarray-Detectable Imbalances

Microarray testing (or other molecular methodology) casts a wider net, and we mention only a very few of the rapidly growing list of examples in which parental transmission is known. Yu et al. (2008) identified two families with a duplication of the DiGeorge region, that is, dup(22)(q11.2): in one family of size 3 Mb, and in the other, 1.5 Mb. The phenotypes differed quite widely through each family, and indeed in one, the normal grandmother acted as carer for two of her disabled grandchildren. A little telomeric of this site is the microduplication of 22q11.21q11.23, most cases of which turn out to have been inherited from a parent, and most of these parents are judged to be “apparently normal” (Coppinger et al., 2009b).The Williams syndrome region, 7q11.23, is another which, when present as a duplication, is not infrequently observed in parent–child transmission (Van der Aa et al., 2009). A number of other examples are on record of variable phenotype, and of inheritance from normal parents (Wentzel et al., 2008; Ou et al., 2008b).

Autism is an area of active research in the microarray laboratory. Microduplication of 16p11.2 is a microarray-level imbalance that has been associated with autism spectrum disorders (Fernandez et al., 2010), although others argue that the associated neurocognitive profile may comprise only nonspecific components that can also be observed in, but not necessarily adding up to, autism (Rosenfeld et al., 2010). This microduplication, and occasionally the countertype microdeletion, can be transmitted from a parent, who may or may not show a cognitive phenotype, and may also be seen in other apparently unaffected family members. The dup(15)(q11.2q13), as mentioned earlier, has an association with autism, and familial autism with a segregating microduplication in this region is described in Van der Zwaag et al. (2010); but the complexity of interpretation of these small genomic lesions is illustrated by the fact that this same microduplication is also seen in a general population, albeit at a lower frequency than in autistic patients. By no means is this the only such example (among many others, see also del(1)(q21.1), p. 311), and interpreting the parental transmission of a genomic imbalance that might or might not be pathogenic is turning out to be a particular challenge as microarray analysis becomes widespread.

A familial phenotype restricted to a single trait, or traits restricted to a single system, might suggest the imbalance of a single important gene, and if this is due to deletion or duplication, molecular analysis may be revealing. Thus, Veenma et al. (2010) studied a large family in which a 15q26.3 microdeletion cosegregated with short stature (the methodology in this case being MLPA). The only notable gene deleted was *IGFR1*, a growth-related gene. The family described in Heron et al. (2010) manifested dominantly inherited neonatal seizures and intellectual disability, and these workers identified a 1.6 Mb duplication at 2q24.3, this region containing a “gene family” of three sodium channel loci.

Triplication.

The rare case of a transmitted triplication is exemplified in Wang et al. (2004), in their report of a mother and her three sons all with 46,trp (4)(q32.1q32.2), and all displaying neurocognitive compromise and minor dysmorphism. Two of the boys had brain imaging, which showed increased size of the ventricular spaces, denoting a reduction in brain substance.

Mosaicism

If a normal cell line coexists with an abnormal karyotype—in other words, there is a 46,N/46,(abn) mosaic state—a high risk for abnormal pregnancy may be implied, an essentially normal parental phenotype notwithstanding. Yip et al. (1996) describe a normal woman with 46,XX/46,XX,der(6)t(6;8)(q27;q22.2), who had presented with recurrent

miscarriage. A balanced 6;8 translocation had presumably arisen at a somatic mitosis, and subsequently an unbalanced der(6) cell line was generated. This cell line likely contributed to ovarian formation, and the miscarrying pregnancies were due to conceptions from 23,der(6) ova. If the abnormal cell line in the parent is predominant, his or her phenotype will be abnormal, as exemplified by the mother reported in Cox et al. (2002) having the karyotype 46,XX,dup(7)(p15.3p22)[75]/46,XX[15], and whose abnormal son had the duplication in nonmosaic state. Very rarely, there can be parental mosaicism with two abnormal cell lines having the opposite imbalance, and a child can be born with one of these imbalances in nonmosaic state (de Pater et al., 2003b).

Parental Partial Aneuploidy due to Small Supernumerary Marker Chromosome or Extra Structurally Abnormal Chromosome (47,+sSMC, 47,+ESAC)

The concept and nomenclature of the SMC has evolved along with the increasing precision which, over the decades, the cytogeneticist has been able to apply to chromosome analysis. The description “marker” originally denoted an additional chromosome whose origin could not be determined. With banding, larger markers could be identified as being, typically, autosomes from which a substantial part had been deleted, sometimes as the result of a rearrangement with another chromosome. Thus, a karyotype written in the 1960s as 47,+mar might come to be recognized, in the latter twentieth century, as, for example, 47,+der(13), 47,+rea(22), 47,+i(9p), 47,+r(8), or 47,+inv dup(15). With yet subtler ability to dissect out the component parts of these chromosomes, only the much smaller ones remained as a challenge in identification. The expression now mostly in use is “small supernumerary marker chromosome” (sSMC), which Liehr et al. (2004) define thus: “structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional banding cytogenetics alone, and generally are equal in size or smaller than a chromosome 20 of the same metaphase spread.” Those supernumerary chromosomes that *can* be identified and characterized unambiguously, we simply refer to as “der,” according to that identification and character (ISCN, 2009).[1]

Some sSMCs produce an abnormal phenotype but many, more than half, do not (Liehr et al., 2006). The actual content of the chromosome will determine its pathogenicity or otherwise. Those containing acrocentric short arm and pericentromeric material, or other autosomal pericentromeric chromatin, are typically harmless (Callen et al., 1992). Liehr and colleagues maintain a Web site of sSMCs containing pericentromeric euchromatin, distinguishing those with and without phenotypic consequence (http://www.med.uni-jena.de/fish/sSMC/ 00START.htm). The sSMC with a normal phenotype might nevertheless be the basis for male infertility, as the additional chromosome may interfere with the meiotic process. In a study of 1000 men with nonobstructive infertility, Elghezal et al. (2006) found one man with a supernumerary marker, although not much weight can be put on a single case (see also section on “Details of Meiotic Behavior”).

One of the largest family studies concerning an sSMC is described in Bakshi et al. (2008). A 50-year-old woman had her chromosomes tested because she had a tongue cancer, and the entirely fortuitous discovery of a very small marker was made. FISH and whole-chromosome painting showed is to be of chromosome 21 origin. In her family, of 11 normal individuals karyotyped, four had the same sSMC, which was seen, as in her, in mosaic state. This tiny remnant of chromatin had tagged along, transmitted through these several meioses, somewhat incontinently surviving mitosis (that is, generating mosaicisms), and all the while completely harmless.

Crolla (1998) reviewed the published studies of supernumerary chromosomes from 1991 to 1998, numbering some 168 cases. The list is exhaustive, and every chromosome other than chromosome 5 is represented (Masuno et al., 1999, subsequently completed the list with their case of 47,XY,+mar(5)/ 46,XY). Many were associated with phenotypic abnormality, in some severe; some ascertained at prenatal diagnosis eventually proved to be normal. Given the wide range of cytogenetic heterogeneity, of course it is completely unsurprising that there should be a wide phenotypic range, and including normality. Each chromosome needs to be assessed on its merits.

As well as the direct analysis of the marker itself, a wider family study can clarify the interpretation. Thus, Takeno et al. (2004) describe a four-generation pedigree, the great-grandmother and the grandmother having the karyotype 46,XX,t(4;13)(p16;q11), with one of the derivative chromosomes, the der(13), being very small. (This chromosome is very similar to the der(13) illustrated in Fig. 5–12). Two sons in the next generation had a tertiary trisomy and a tertiary monosomy, respectively; and the son with the tertiary trisomy, 47,+der(13), then went on to transmit the very small derivative—a chromosome formerly known as “marker”—to his daughter.

A supernumerary chromosome that could be pathogenic in nonmosaic form might, in the mosaic state, have no apparent phenotypic consequence. For example, Rothenmund et al. (1997) identified a sSMC in a father as derived from the pericentromeric region of chromosome 8, he having the mosaic karyotype 46,XY[90]/47,XY,+der(8)[10], while his two mentally handicapped daughters had a (nonmosaic) karyotype 47,XX,+der(8).

Some particular chromosomes contribute more to the sSMC population than others, one such being chromosome 22. Crolla et al. (1997) propose that der(22) markers can be sorted into those with and those without euchromatin, the former typically being with phenotypic consequence, and the latter not. Urioste et al. (1994b) describe a familial unstable supernumerary chromosome with a mother and two daughters having variable manifestations of cat-eye syndrome due to 46,N/47,+der(22).

Partial autosomal aneuploidy resulting from a ring chromosome, as the supernumerary chromosome, is dealt with in Chapter 11. (This separation into chapters, while pragmatic clinically, may not be entirely appropriate, given that many very small markers, subjected to a fine molecular dissection, may turn out actually to be rings; Baldwin et al., 2008.)

Details of Meiotic Behavior

The supernumerary chromosome would probably form a univalent at meiosis rather than synapsing with whatever chromosome from which it was derived. Martin et al. (1986a) analyzed sperm chromosomes from two men who had bisatellited markers. Slightly less than half the sperm were found to carry it, although the distribution did not differ significantly from 1:1. However, Cotter et al. (2000) showed a much reduced frequency of sperm carrying a marker, 6% compared with an expected 50%, in a phenotypically normal man with 47,XY,+del(15)(q11.2).

Very small markers are prone to loss during cell division. Consequently, mosaicism and, in the case of a transmitted chromosome, familial mosaicism are seen frequently (Chudley et al., 1983). Adhvaryu et al. (1998) describe a bisatellited 15-derived SMC in only 2% of cells in a grandfather, presumed of postzygotic origin in him, whose daughter and a grandson were nonmosaic 47,+SMC, with the chromosome evolving in another grandchild into a very small ring. All these people were phenotypically normal.

Familial SMCs are characteristically maternally transmitted, which could reflect preferential exclusion of the marker in spermatogenesis or, in some, a reduced male fertility, as noted earlier. Jaafar et al. (1994) studied a phenotypically normal man presenting with infertility who had a supernumerary bisatellited heterochromatic chromosome. In most spermatocytes, the marker was in close proximity to the X-Y bivalent, and this may have been the cause of the infertility.

Genetic Counseling

Parental Trisomy 8 or Trisomy 18 Mosaicism

The very rare individual for whom genetic counseling may be a practical issue is likely to have a low-grade mosaicism, and the diagnosis may have been made because of reproductive loss. Recurrent miscarriage may reflect a higher fraction of trisomic tissue in the gonad, and the risks for a further pregnancy may be substantial. Wei et al. (2000) undertook preimplantation genetic diagnosis in one case, an infertile man with mosaic trisomy 18.

> A separate issue relates to the possible risk for hematological malignancy in individuals with trisomy 8 mosaicism (Seghezzi et al., 1996; Brady et al., 2000). Trisomy 8 is well known as an acquired change in the cascade of carcinogenesis in myeloid neoplasias (particularly myeloid leukemia, myelodysplastic disease). It may be that constitutional trisomy 8 acts similarly, in this case as the “first hit,” in those individuals in whom the trisomic cell line includes the marrow (as of course it must in patients diagnosed on a peripheral blood karyotype). The level of risk for cancer predisposition is unknown. For the myeloid neoplasias, unlike many other cancers, little therapeutic advantage comes from earlier diagnosis, and the counselor will need to consider carefully how, or if, this issue may be raised (see also p. 15).

Parental Trisomy 21 Mosaicism.

Theoretically, the risk for having a child with (nonmosaic) Down syndrome is high, up to 50%. Presumably the risk is related to the proportion of gonadal cells that are trisomic, but this is not accessible information. The proportion seen on lymphocyte analysis offers no real help in this question. One point is clear: it is certainly appropriate to offer prenatal diagnosis. Indeed, preimplantation genetic diagnosis may be warranted, as Conn et al. (1999) attempted with a woman who had presented with a history of three out of four pregnancies with trisomy 21, herself with a nonmosaic normal karyotype on blood. Three of four unfertilized oöcytes were hyperhaploid 21, and four out of six analyzable embryos were trisomic 21 (some mosaic). Transfer of the two chromosomally normal embryos was not successful.

Parental Down Syndrome.

The risk to conceive a trisomic 21 conceptus is presumably 50%, at least in the case of maternal DS. The other parent is likely to have a degree of mental deficiency, possibly due to a genetic factor. Prenatal diagnosis would of course detect those fetuses with trisomy 21, but the risk of a chromosomally normal fetus having a birth defect or being mentally handicapped could be as high as 30% (Shobha Rani et al., 1990). Prevention of pregnancy in those DS women at risk is regarded by many as advisable. In two confirmed cases of paternity in a DS man, prenatal diagnosis (with a normal result in both) was performed (Bobrow et al., 1992; Pradhan et al., 2006). We refer the reader to the discussion in Chapter 1 (p. 17) for a general review of the ethical issues in this setting.

Parental Partial Autosomal Trisomy or Monosomy.

The risk for a child to have the same defect as the parent is 50%, or very close to it. A lesser risk applies in the case of mosaicism. In the case of a person of only low-normal/borderline intelligence who is functionally fertile, the issue of the risk and possible questions of prenatal diagnosis or sterilization will be difficult to raise (p. 17).

A phenotypically normal individual said to have a partial aneuploidy merits a cytogenetic or microarray reevaluation, to check for the possibility that the supposed aneuploidy is actually a balanced rearrangement that was only partially characterized.

As cytogenetic techniques have become refined, new "defects" are being identified which, upon family study, are revealed as being unusual, but functionally balanced, forms (Barber, 2005). For practical purposes, they can be regarded as variants, rare or possibly even "private" to that family. The counselor must take care that apparently abnormal karyotypes are not overinterpreted. In a family study undertaken to clarify a particular unusual chromosome, family members should understand the reason for the study.

The Harmless Supernumerary Marker Chromosome.

Each chromosome needs to be judged on its merits, a detailed cytogenetic assessment and parental karyotyping study having been undertaken. If a SMC is judged to be harmless, then on that basis it would be immaterial whether a parent transmits it to an offspring. Nevertheless, it may be useful to know that, in a particular family, there has not been any associated phenotypic abnormality, a point that may be settled by doing a family study; this enables the counselor to offer firm reassurance that those who inherit it in the future will also likely be normal. A possible association with male infertility is mentioned earlier.

The very rare circumstance of an sSMC identified in a normal person—and seeming, at first sight, harmless, but the sSMC then revealed to have been derived from a deletion on the homolog concerned—is dealt with on p. 158. These carriers have a high risk for abnormal offspring.

Notes:

[1] In cancer cytogenetics, the complexities of rearrangement are such that even a large abnormal chromosome might not readily be identified, as to the nature of its component parts. Here, there word *marker* can continue to be used, whatever the size of the chromosome.







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The Fragile X Syndromes

Chapter: The Fragile X Syndromes
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FRAGILE XA SYNDROME appeared on the cytogenetic scene in the late 1970s, although by the mid-1990s it occupied a predominantly molecular genetic stage. But it is entirely appropriate that it retains a place here, and indeed a whole chapter. Fragile XA syndrome (or simply fragile X syndrome; FXS, in common parlance) is the second most prevalent genetic cause of mental deficiency after Down syndrome, and it is the most common familial cause. The premutational state is associated with a degenerative neurological syndrome typically of onset in late middle age, and, in the female, ovarian insufficiency; and we may speak of the fragile X associated syndromes (FXSs). Fragile XE syndrome is rare, but it is one of the more common forms of nondysmorphic mild intellectual disability.

Biology

Fragile XA syndrome (FXS) is named for the folate-sensitive fragile site, FRAXA, at Xq27.3 (Fig. **15–1**). The first family in which this fragile site was reported was seen, initially, as a fascinating story but of limited practical relevance (Lubs, 1969); some four decades later, the FXDs command a prominent stage worldwide. There are two other rare fragile sites distal to FRAXA, named FRAXE and FRAXF. FRAXE is a rare folate-sensitive fragile site that is the chromosomal manifestation of an expanded CCG repeat that silences the *FMR2* gene, which is the molecular basis of its phenotype of nonspecific mental retardation (Gecz et al., 1996). FRAXF appears to be a harmless rare variant, although the full mutation does silence the *FAM11A* gene (Shaw et al., 2002). FRAXD is a common fragile site, proximal to FRAXA, and it is also harmless and a part of normal chromosome structure.

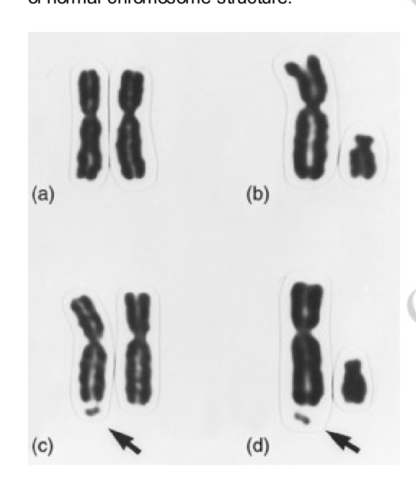


Figure 15–1
Plain-stained sex chromosomes from a normal female (*a*) and male (*b*) compared with those of a fragile X female (*c*) and a fragile X male (*d*). The fragile site is arrowed. The segment distal to the fragile site appears as a satellite, as though it is about to break off.

The FRAXA fragile site exists within the 5′-UTR of the *FMR1* (fragile X mental retardation-1) locus, which encodes a protein named FMRP (fragile X mental retardation protein). FMRP acts as a regulator of mRNA transport and of translation of specific mRNAs at the synapse (Oostra and Willemsen, 2009), and it is necessary for normal brain development and function. The nature of the mutation in FXS is an expansion of a normal sequence of trinucleotide (CCG) repeats within this 5′ region. The different fragile X trinucleotide repeats (*FMR1* alleles) can be grouped into four size categories: common (6–40 repeats), intermediate or gray-zone (41–58 repeats), premutation (59–200 repeats), and full mutation (>200 repeats). The classic clinical picture of FXS due to the full mutation will be familiar to most counselors: intellectual disability, mood disturbance, and abnormal behavior that may merge into frank autism, and physical traits including a subtly distinctive facies, joint hypermobility, and, in the male, macro-orchidism. A child from the original family reported by Lubs (1969) is shown in Figure **15–2**.



Figure 15–2
A child with fragile X syndrome from the family in which the fragile site was first recognized. The facies is characteristic. (From H. A. Lubs, 1969, A marker X chromosome, *American Journal of Human Genetics* 1969;21:231–244. Courtesy H. A. Lubs; reproduced with the permission of the American Society of Human Genetics.)

Incidence

The *full mutation* exists in 1 in 4000–5000 males, as measured from indirect and direct approaches, an example of the latter being a study of 36,124 newborn males having routine bloodspot screening (Crawford et al., 2001; Coffee et al., 2009). An important figure is the frequency of the *female premutation* carrier, these women being at risk to have a child with FXS. Somewhat different figures have been derived from different populations. In Quebec, about 1 in 260 women carry a premutation of 55–101 copies of the CCG repeat (Rousseau et al., 1995), while in Finland the fraction is very similar at 1 in 250, for women having more than 60 copies of the repeat (Ryynänen et al., 1999). In Israel, the figure is higher, and in Japan, less. One in 150 Israeli women have 55 or more repeats (Berkenstadt et al., 2007), while in Japan the incidence of fragile X appears to be lower than in other populations, and Otsuka et al.(2010) found no premutation carriers among 576 men and 370 women, albeit that these denominators were rather small.[1] In theory, the *male premutation* carrier prevalence should be close to one half of the female rate. One estimate is 1 in 1000 (Crawford et al., 2001), although this figure is to be seen alongside the 1 in 250 fraction that Fernandez-Carvajal et al. (2009b) derived from a study of 5267 male newborns having blood spot screening in northwestern Spain. For the record, in this Spanish study, the frequency of the *intermediate (gray-zone) repeat* in the male was 1 in 26. Castellví-Bel et al. (2000) showed that the incidence of intermediate *FMR1* alleles was not significantly different in retarded and in appropriate control populations, although recent suggestions of a possible morbidity association are noted later.

Intellectual disability associated with FRAXE is rare and probably affects less than about 1 in 100,000 individuals (Gecz, 2000). Sporadic patients with FRAXE may not be routinely diagnosed, testing only being performed when there is a family history of intellectual disability under investigation.

Molecular Genetics of the Fragile X Loci

Fraxa (FMR1)

Fragile XA syndrome is a "dynamic mutation" disorder (Sutherland and Richards, 1993). As outlined earlier, there is a section of DNA at the fragile site comprising the triplet cytosine-cytosine-guanosine (CCG) repeated many times.[2] The number of repeats determines the genotype of normality, "gray zone," premutation, or full mutation (Table 15–1). Normal X chromosomes have from 6 to about 58 sequentially repeated copies of the CCG triplet, with copy numbers between 40 and 58 being regarded as in a "gray zone." Hemizygous carrier males with the premutation have approximately 58–200 copies of the triplet-repeat, as do female heterozygotes on one of their X chromosomes. Males with the "full mutation" of more than 200–220 triplet-repeats (up to about 1000 copies) have FXS. The situation is a little more complex for females with the full mutation, reflecting variable Lyonization in the brain, with some neurons expressing the normal, and some the abnormal chromosome. Of female carriers with the full mutation, about half have some degree of intellectual disability, but rarely of a similar degree to the male (Thompson et al., 1994).

Table 15–1. Trinucleotide Lengths and Associated Phenotypes

NO. CCG REPEATS	GROUP	MALE COGNITIVE PHENOTYPE	FEMALE COGNITIVE PHENOTYPE
6–39	Normal	Normal	Normal
40–54	"Gray zone"	Normal	Normal
55–200	Premutation	Normal[a]	Normal[b]
>200	Full mutation	Mentally retarded	Variable

[a] Possible cognitive impairment and fragile X tremor/ataxia syndrome in later middle age (see text and Table 15–2).

[b] Some may show a mild psychological/personality effect; and there is an association with premature ovarian failure and fragile X tremor/ataxia syndrome (see text and Table 15–2).

The CCG triplet-repeat sequence, when increased beyond a critical size (in the vicinity of 55 copies), is unstable and can change in copy number when transmitted from parent to child. Thus, a mother with a premutation can transmit a full mutation to her child. A mother with a full mutation can transmit a larger or smaller full mutation to her child. A mother with a "gray zone" allele can transmit an expansion of this allele to her child, into the premutation range, but not as a full mutation. In contrast, fathers only transmit premutations to their children (necessarily only their daughters) regardless of whether they themselves carry a premutation or, very rarely, a full mutation. (While very rare exceptions to this have been reported, e.g., Zeesman et al., 2004, they can usually be disregarded in practice.) The change from normal copy number to full mutation is a multistep process, proceeding through premutation steps presumably over a number of generations, rather than as a single event characteristic of classical mutation. The likelihood of expansion is related to the presence of "AGG interruptions" (or CCT[2] interruptions) in the premutation repeat: the longer the run of pure CCGs, the greater the propensity for expansion (Nolin et al., 2011).

Laboratory Analysis.

The varying sizes of the fragile site DNA can be visualized on a Southern blot. Figure **15–3** shows the patterns seen in various types of individual and demonstrates the instability in a family, and further examples are shown in Figure **15–4**. If DNA is digested with the enzyme *Pst*I and probed with pfxa3, the normal X chromosome gives a fragment of approximately 1.0 kb. This ~1.0 kb band represents about 900 bp of DNA flanking the repeat plus the 18–165 bp of the actual CCG repeat sequence itself (which amounts to 6–55 triplet-repeats). The DNA fragment from a fragile XA chromosome, having within it the additional copies of the CCG repeat sequence, is larger by this amount. For example, in a person with 200 copies (200 × 3 = 600 bp) of the triplet-repeat—which is about where the premutation merges into the full mutation—the fragment is ~1.6 kb (~1000 bp + 600 bp) in size. This is an increase ("amplification") of ~600 bp over the normal size of 1 kb. Basehore and Friez (2009) outline a protocol for the complementary application of polymerase chain reaction (PCR) and methylation-sensitive Southern analysis.



Figure 15–3
Inheritance of the fragile XA unstable element in a four generation lineage from a large affected pedigree. Pedigree symbols: normal carrier female expressing the fragile XA on cytogenetic study = ◑; affected FXS male expressing the fragile XA = ■; normal female = ○. All carriers, ⊙ and ⊡, are obligate carriers. Chromosomal DNA was digested with *Pst*I and probed with pfxa3. The control probe pS8 was included in the hybridization.

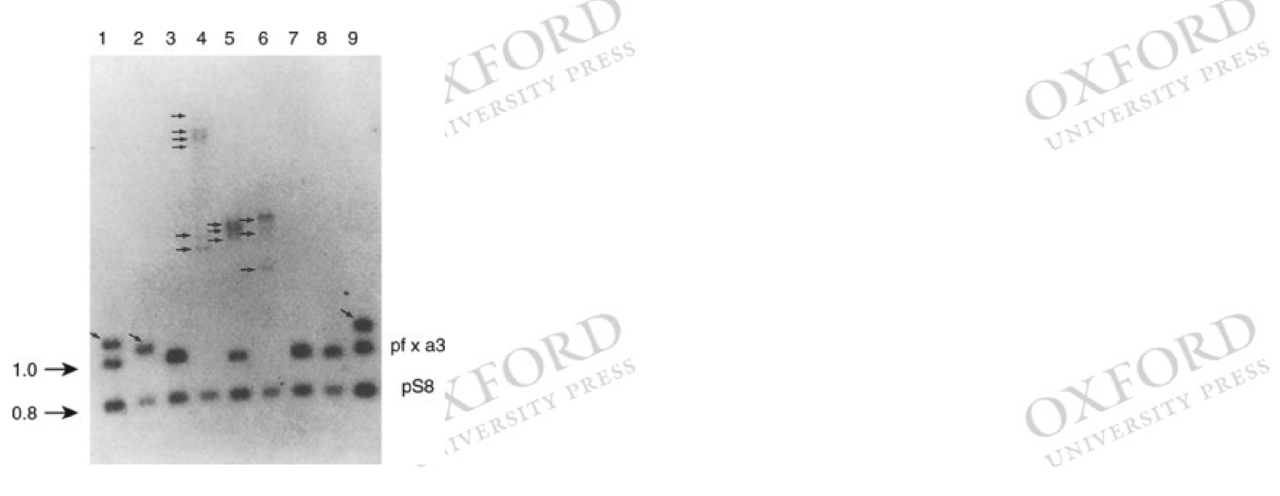


Figure 15–4
Southern blot characteristics of different genotypes. (1) Carrier female, (2) transmitting male, (3) noncarrier female, (4) affected male, (5) carrier female, (6) affected male, (7) noncarrier female, (8) normal male, and (9) carrier female. PstI-digested DNA probed with pfxa3 and pS8.

Full Mutation: Methylation of The *FMR1* Gene

Hypermethylation has a central role in the mechanism by which the large-size CCG repeat (>200 repeats) causes the molecular pathology that, in turn, leads to the FXS phenotype. The repeat sequence is located within a noncoding portion (the 5'-untranslated region) of the *FMR1* gene. Once there are more than about 200 copies of the repeat, the DNA surrounding the repeat, and the repeat itself, become hypermethylated. (Methylation is the addition of methyl groups to the C (cytosine) bases in CpG sequences in the DNA molecule.) Hypermethylation is associated with inactivation, in *cis*, of the *FMR1* gene containing the triplet-repeat sequence, and it ceases to be transcribed: a "*loss-of-function*" mutation. The consequential lack of the *FMR1* gene product (FMRP) is the cause of the abnormal phenotype of the FXS. The normal function of FMRP is to control the activity of proteins involved in the pre- and postsynaptic machinery of the central nervous system; absence of FMRP leads to dysregulation of these proteins, and thus compromise of brain functioning.[3] A specific observation in brain tissue from patients with FXS is an excess of immature forms of the "spines" that project from the branches (dendrites) of the neurons (Irwin et al., 2001), and which comprise the physical basis of synapses (connections between neurons). Particular regions of the brain may be susceptible, and Haas et al. (2009), using sophisticated imaging analyses, point to certain white matter tracts that connect the left frontal lobe to deep structures (the ventral frontostriatal pathway), and which contribute to the control of appropriate inhibitory behavior, as having an abnormal fiber density.

Determination of repeat copy number and, if necessary, assessment of methylation as an indicator of the activity state of the gene, can be used to help predict phenotype. Methylation status of the triplet-repeat and adjacent DNA in affected males cannot be resolved from the routine *FMR1 Pst*I digestion DNA test (see later discussion). "High-functioning" affected males may have amplification values of between 0.6 and 1.0 kb above the normal size. If a more accurate genotype-phenotype correlation is required, analysis of methylation status is done by probing a double digest of *Eco*RI and *Eag*I. *Eco*RI/*Eag*I double digests of DNA from males within this range of amplification values and probed with pfxa3 are shown in Figure **15–5** (the probes StB12.3 and Ox1.9 would give an identical result). Interpretation is simpler in males than in females. The normal chromosome of carrier females is also methylated in half the cells, on average, as part of the normal random X-inactivation process.

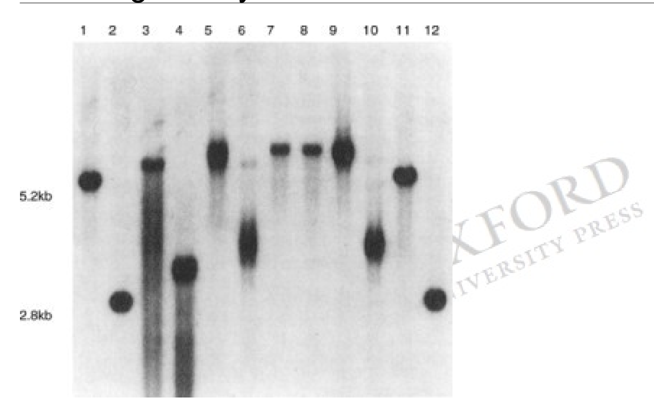




Figure 15–5
Restriction patterns for fragile X syndrome males who show methylation differences. EcoRI digests are shown in odd-numbered lanes; *EcoR*I/*Eag*I double digests are shown in even-numbered lanes. Normal control male, lanes 1–2 and 11–12. The normal *EcoR*I fragment is 5.2 kb and the normal unmethylated *EcoR*I/*Eag*I fragment is 2.8 kb. Lanes 3 through 10 for affected individuals show fragments of higher-than-normal molecular weight because of amplification. (Females would exhibit additional complexity because additional methylated and unmethylated bands occur for the normal X chromosome.) The affected individual in lanes 3–4 is unmethylated. Lanes 5–6 is a methylation mosaic; most of his cells have the *Eag*I restriction site unmethylated, but in some cells it is methylated. The individual in lanes 7–8 is fully methylated, and the one in lanes 9–10 is unmethylated. The tissue tested was blood, which may not necessarily reflect methylation patterns in other tissues. (From D. Z. Loesch et al., 1993, Genotype-phenotype relationships in fragile X syndrome: a family study, *American Journal of Human Genetics* 53:1064–1073. Reproduced with the permission of the American Society of Human Genetics.)

While methylation may have a role, it is not the only process influencing the function of *FMR1*. In the absence of methylation, the fully expanded (>200 repeats) gene is overtranscribed, but the mRNA is inefficiently translated, and the degree of inefficiency increases in proportion to repeat copy number. Feng et al. (1995) analyzed clones of cells having 207, 266, and 285 repeats: these produced, respectively, 24%, 12%, and essentially 0% of the normal amount of FMRP. Possibly, this reduction in protein is cell type specific and certain tissues (for example, chorionic villi) with large unmethylated repeats can still make some FMRP.

Premutation: RNA Toxicity

The *FMR1* premutation[4] has its pathogenic effect due to an entirely different mechanism: a toxic *gain*-of-function. The expanded (55–200 repeats) *FMR1* gene is transcribed, and in increased amounts; and these enlarged RNA transcripts then behave abnormally. They aggregate within the nucleus of the neuron, and these aggregations attract certain other species (e.g., lamin A/C, αB-crystallin). These several substances are sequestered as intranuclear inclusions. Over time, these inclusions compromise the functioning of the neuron, and thus the functioning of the various component parts of the neural substrate in which this process is taking place (Greco et al., 2002; Brouwer et al., 2009; Gokden et al., 2009). Some other classes of cell are also affected, for example, the hormone-producing cells of the testis and thyroid; and a similar process affecting the ovary is likely the basis of the premature ovarian failure in the female (Jacquemont et al., 2007).

The Effect of FMR1 Premutations on Carrier Males

Neuropsychological Capacity

Many men may be free of neuropsychological complication (Hunter et al., 2010), but a few children with high-end premutations may manifest learning difficulty. Some premutation males may display deficits of executive functioning and memory on neuropsychological examination (Moore et al., 2004). Men over 50 with larger (70–200) repeats have a six-fold increased risk for a progressive cognitive impairment (Sévin et al., 2009).

The Tremor-Ataxia Syndrome.

The *FMR1* premutation can lead to a neurodegenerative disorder, first described in 2001: the “fragile X tremor/ataxia syndrome” (FXTAS) (Hagerman et al., 2001; Brouwer et al., 2009). The most severe involvement is expressed as a progressive neurological syndrome with cerebellar and parkinsonian features, accompanied by decline in executive function and cognitive capacity, with overt onset usually in late middle age. A consistent neuropathological pattern, with widespread neuronal intranuclear inclusions, has been shown at postmortem. The cerebellar neuroradiology is characteristic and may precede actual onset of symptoms (Brunberg et al., 2002; Loesch et al., 2008).

The penetrance of FXTAS is in the range of 33%–45%, and thus about a third to almost a half of premutation males will develop this condition, in an age-dependent and repeat size-dependent manner (Jacquemont et al., 2004; Rodriguez-Revenga et al., 2009). The smallest premutation to result in FXTAS is 69 repeat copies. The risk in Sévin et al. (2009) was greater (33%) for midsize/large (70–200 CCG) repeats than for smaller ones (55–59 CCG), the penetrance in the latter group of 5.9% being close to the 5.1% control figure; this study also noted that cognitive impairment preceded the onset of the actual syndrome. Penetrance estimates are set out in Table 15–2.

Table 15–2. Penetrance of Fragile X Tremor/Ataxia Syndrome in Premutation Carriers, Shown as Percentage According to Age

AGE (YEARS)	SERIES		
	JACQUEMONT ET AL. (2004)	RODRIGUEZ-REVENGA ET AL. (2009)	
	MEN	MEN	WOMEN
50–59	17% (12)	0% (1)	4% (27)
60–69	38% (13)	53% (17)	20% (15)
70–79	47% (17)	53% (15)	26% (19)
> 80	75% (4)	27% (11)	21% (24)

Note: The reduction in penetrance in the >80 group in Rodriguez-Revenga et al. likely reflects increased mortality in the affected individuals. Numbers of subjects shown in parentheses.

The Effect of FMR1 Premutations on Carrier Females

Neuropsychological Capacity.

Typically, females who carry *FMR1* premutations have intelligence within the normal range, but some lines of evidence point to subtle effects, and sometimes more overt psychopathology, in some carriers (Bourgeois et al., 2009). Depression is more frequent, and may be more marked with larger repeats, above 100 CCG copies; it is controversial whether this association might have an intrinsic neurological basis or be secondary to ovarian hypofunction (Hunter et al., 2010). Migraine is common.

The Tremor-Ataxia Syndrome.

In the initial study of Jacquemont et al. (2004), the incidence of FXTAS symptoms in 59 premutation women was not greater than in controls. However, the same group (Coffey et al., 2008) subsequently found a penetrance of 8% in 72 premutation carriers aged greater than 40 years. Rodriguez-Revenga et al. (2009) showed that 16% of such women aged 50 or more years (rising to 22% of those over 60 years) had signs of FXTAS.

Ovarian Insufficiency.

Ovarian function is affected, with consequential primary ovarian insufficiency/senescence (POI/S), which may then proceed to actual premature ovarian failure (POF). Allingham-Hawkins et al. (1999) showed that among 395 premutation carriers, 16% had early (before age 40) menopause, compared with only 0.4% in a control group comprising noncarrier relatives. Hundscheid et al. (2000) give likelihoods for POF according to age as follows: 4% by age 30 years, and 25% by age 40 years. The risk for POI/S and for POF correlates with the number of CCG repeats (Sullivan et al., 2005; Hunter et al., 2008b), and a risk for POI/S may also apply to women carrying a gray zone allele, and even an upper-range normal allele (Gleicher et al., 2009; Streuli et al., 2009).

Other Complications.

More broadly, the carrier state in the female is associated with a range of other medical problems, including in particular thyroid disease and fibromyalgia (penetrances of 16% and 24%, respectively), and with seizures, high blood pressure, and peripheral neuropathy also recorded (Coffey et al., 2008; Rodriguez-Revenga et al., 2009; Chonchaiya et al., 2010).

Intermediate (“Gray-Zone”) alleles

To add further complication, triplet expansions intermediate in size between the accepted “common” range (up to ~40 repeats) and the onset of premutations (~58 repeats) might possibly convey a phenotype, although this is controversial. As noted earlier, premature ovarian insufficiency/senescence may develop in the female heterozygote for a gray-zone allele. A case for cognitive or behavioral compromise, which may present as autism, in a child of either sex, has been proposed (Loesch et al., 2007). Likewise a suggestion has been made of an increased risk for parkinsonism (Loesch et al., 2009; Zhang et al., 2009b), but this remains to be confirmed (Kurz et al., 2007). A single instance of expansion from a gray zone allele to a full expansion is on record: from grandfather (52 repeats) through his daughter (56 repeats) to his severely retarded grandson (~538 repeats); the grandfather displayed tremor and ataxia (Fernandez-Carvajal et al., 2009a). This is the first report of an allele of <59 repeats expanding to a full mutation in a single step.

Two Types of FMR1 Mosaicism.

Individuals with full mutations can show somatic instability of the amplified repeat sequence. Different cells in a single tissue can be genetically different, in terms of triplet-repeat length; and genetic differences can exist between tissues. This is manifest as a smear of DNA fragments on Southern blot (Fig. **15–4**, lanes 4–6).

This instability can lead to two types of mosaicism. While strictly speaking individuals with smears of DNA in the full mutation size range are mosaics (there are different lengths of triplet-repeats), this term is reserved for two specific situations.

First, in *mutational mosaicism* there are some cells with full mutations, which are fully methylated; and some cells with premutations, which are unmethylated and the gene can function. The mental phenotype can vary, presumably depending upon the type of mutation predominating in different parts of the brain, and thus the regional activity within cerebral tissues of the *FMR1* gene. Up to 20% of FXS males are mutational mosaics detectable on Southern analysis and some may have an IQ within, but at the lower end of, the normal range. A rare type of mutational mosaic is the individual with some cells containing a normal number of repeat copies and others containing premutation or full mutation copy numbers. Polymerase chain reaction is a more subtle tool and may indicate the presence of a premutation in a very small fraction of cells in many FXS males. De Graaff et al. (1995) could show on brain tissue from an affected male that about 1% of neurons expressed FMRP, and thus presumed that these individual neurons had a genetically active premutation.

Second, in *methylation mosaicism*, the number of repeat copies is characteristic of a full mutation, but the DNA is not methylated in all cells (Fig. **15–5**, lane 6). This is less common than mutational mosaicism and occurs mostly at the lower end of the range of full mutation copy number. “High-functioning” FXS males may be methylation mosaics, with full mutations which are partly or completely unmethylated.

Other Mutational Bases of FRAXA.

Almost all FXS is due to triplet-repeat expansion. A very few affected persons have a different mutational basis, and deletions (ranging in size from 1 bp to about 100 bp and up to megabase size) and point mutations in the *FMR1* gene have been reported (Hammond et al., 1997; Grønskov et al., 2011). Duplication of a 5 Mb segment within Xq27.3q28 and which includes the *FMR1* locus, as well as *FMR2* and 26 other genes, leads to a heritable syndrome of mental retardation and short stature with hypogonadism (Rio et al., 2010). These authors propose that a functional disomy of *FMR1* contributes to the clinical phenotype. Grønskov et al. (1998) and Castellví-Bel et al. (1999) screened a number of patients with a high clinical score for the features of FXS, and yet found no mutations.

Fragile XE

The mechanism is again a CCG repeat that expands through premutations to full mutations (Mulley et al., 1995). The CCG repeat is in the 5´ untranslated region of a gene called *FMR2*, whose 9.5 kb transcript encodes a putative 1302 amino acid protein. *FMR2* transcription is silenced in males with CCG expansion with methylation of the adjacent CpG island (Gecz et al., 1996). Because of its milder phenotype, and because it is so rare, the diagnosis is not often clinically suspected, although it is one of the more common forms of nonsyndromal mild X-linked mental retardation. In some FRAXE families there will be males who have full mutations who are not intellectually disabled, as the cognitive impairment associated with FRAXE overlaps the normal range (Gecz 2000). Microdeletion within the *FRAXE* gene has been identified on microarray (Sahoo et al., unpublished data), and it may be that further such cases will be recognized with the increasing use of molecular methodology.

Fragile XF.

FRAXF is also due to dynamic mutation of a CCG repeat (Parrish et al., 1994), but in this case an apparently harmless one, in that it is not associated with a familial form of intellectual disability.

Cytogenetics

The appearance of the X chromosome in FXS is now of essentially academic interest. But historically, this is what gave the syndrome its name, and the counselor should be aware of this history. The “fragility” refers to a fragile site on the chromosome at which the chromatin is attenuated, and which resembles a gap or break (although there is in fact usually continuity) (Fig. **15–1**). In the early days of the syndrome, laboratory technology was developed that stressed the chromosomes, and this was essential for the fragile site to manifest itself and to be seen by the cytogeneticist (Sutherland, 1991). These induction methods all led to a relative deficiency of either thymidine or deoxycytidine at the time of DNA synthesis. It is of interest that the fragile site was seen in almost all affected FXS males and most females, and in most carrier females who had the full mutation, but not in carriers (male or female) with a premutation. As well as the FRAXA fragile site, other sites within distal Xq are FRAXE, and the normal variants, FRAXD and FRAXF (see earlier).

Genetics

FMR1

The inheritance of FXS is certainly X linked, but it becomes a matter of semantics as to whether this might be “X-linked semidominant,” or “X-linked incomplete recessive.” More usefully, we may refer to the premutational or full mutational state, to the associated gender-specific penetrances, and to the probability of expansion from the premutational to full mutational state upon transmission of the chromosome to a child. The following points may be noted.

De Novo Full Mutations Do Not Happen

No new mutation has been observed; that is, no individual with a full mutation has been observed as the offspring of parents who have normal numbers of copies of the CCG repeat on their X chromosomes. The mothers of all FXS individuals are carriers of at least a premutation, and where study has been possible, so is a grandparent. The rate of mutation from normal CCG copy number to premutation thus appears to be very low.

Male Transmission.

When the unstable sequence is transmitted by males, it characteristically does not increase in size, and it may decrease. In males with full mutations, only premutations are seen in sperm (Reyniers et al., 1993). (There are extremely rare exceptions to this rule: Zeesman et al. (2004) reported a male who was a premutation/full mutation mosaic whose daughter was also a premutation/full mutation mosaic.)

Female Transmission.

When the unstable triplet-repeat sequence is transmitted by females, it usually increases in size (although rare decreases have been reported, and even more rarely, gene conversion events have reduced mutation-range fragile X chromosomes back to normal; Losekoot et al., 1997). Women who transmit X chromosomes with small premutations usually show less increase in size than women who transmit larger premutations. Thus, women with fewer than 70 triplet-repeats (“low-end” premutations) mostly have children who also have premutations, although the premutations in these sons and daughters are characteristically larger than those of their mothers. On the other hand, women with “high-end” premutations (90 or more triplet-repeats), and carriers who themselves have a full mutation, almost always transmit a full mutation (Table 15–3). The risk is influenced by whether there are “AGG interruptions” in the premutation repeat sequence (see earlier), and this may be the molecular basis of the observation that the risk for expansion is greater in those women who have a known family history of FXS, versus those ascertained by other means (e.g., through a screening program) (Nolin et al., 2011).

Table 15–3. Risks for Fragile X Premutation Carriers to Have a Child with a Full Mutation, If the Fragile X Chromosome Is Transmitted

PREMUTATION SIZE (TRIPLET-REPEATS, *n*)	OFFSPRING WITH FULL MUTATION/ALL FRA(X) PREGNANCIES	RISK FOR FULL MUTATION IF FRA(X) TRANSMITTED (%)
Nolin et al. (2003)		
55–59	1/27	4
60–69	6/113	5
70–79	28/90	31
80–89	81/140	58
90–99	89/111	80
100+	194/197	98
Berkenstadt et al. (2007)		
55–59	0/79	0
60–69	3/77	4
70–79	4/9	44
80–89	6/11	55
90–99	2/2	100
100+	13/13	100
Nolin et al. (2011)		
45–49	0/55	0
50–54	0/51	0
55–59	0/86	0
60–69	2/81	2
70–79	15/47	32 (54%; 11%)*
80–89	45/61	74 (88%; 33%)*
90–99	31/33	94
100+	93/95	98

Note: The risk becomes higher with a larger expansion size in the mother; the risks are less for mothers ascertained other than through having a family history of fragile X syndrome.

* The % figures in parentheses for allele sizes 70–79 and 80–89 from Nolin et al. (2011) show the higher risks for mothers with a family history of fragile X syndrome (54%, 88%), versus lower risks for women ascertained otherwise (11%, 33%).

Sources: Data are from three large studies: Nolin et al. (2003), Berkenstadt et al. (2007), and Nolin et al. (2011). The former group was ascertained through an affected fragile X syndrome individual, and the risk figures appropriately adjusted; the latter two are based upon prenatal diagnostic data. In Berkenstadt et al. (2007), the women were recruited prospectively as part of a broad screening program; in Nolin et al. (2011), a retrospective analysis was undertaken from multicenter data.

FRAXE (*FMR2*)

Not enough fragile XE families have been reported in detail for segregation analyses to be performed. Notably, and distinct from FRAXA, instances of a FRAXE full mutation being passed from father to daughter are on record (Hamel et al., 1994; Carbonell et al., 1996).

Rare complexities

Bringing together the two Fragile X syndromes, we have seen a family in which both FRAXA and FRAXE are segregating (Mulley et al., 1995). A FRAXE man married a FRAXA heterozygote, and they had two daughters, one a FRAXE heterozygote and the other a FRAXA/FRAXE compound heterozygote. The latter, in turn, has had a son with FXS, and *FMR2* and *FMR1* carrier daughters. There are cases reported of women who are *FMR1* compound heterozygotes, having a full mutation on their maternal X, and a premutation on the X from their father (Linden et al., 1999; Hegde et al., 2001).

The fragile X phenotype can coexist with other abnormalities as part of a contiguous gene syndrome, or simply by chance. Quan et al. (1995) report a child in whom a deletion in Xq26.3–27.3 removed the *FRM1* and adjacent loci. His phenotypically normal mother showed selective inactivation of the deleted X. A mother described in Missirian et al. (2000) was heterozygous for an *FMR1* premutation and a 22q11.2 deletion. Two of her children had DiGeorge syndrome (one also with a *FMR1* premutation), and one had FXS with a full mutation.

Diagnosis.

As outlined earlier, laboratory investigation is typically based upon PCR amplification of the CCG repeat and sizing by capillary electrophoresis. This can be used for both *FMR1* and *FMR2*. Males without a clear product in the normal size range, and females who are not clearly heterozygous for two normal alleles, should then be studied by Southern blot analysis for amplification of the repeat. For prenatal diagnosis, Southern blot is the gold standard, when PCR cannot demonstrate normal parental allele(s) in the fetus.

Screening for Carrier Status in Women of Reproductive Age.

The prevention of FXS, in further pregnancies in a family where an affected child has been identified, is "secondary prevention." Primary prevention would require that carrier women be detected ahead of having a child with fragile X: in other words, in families without a proband. Toledano-Alhadef et al. (2001) describe their experience of offering screening to women in Israel. They tested 14,334 women without any family history of mental retardation and found 3 full mutation carriers and 127 premutations of 55 repeat copies or more. Among the premutation carriers who were, or who became pregnant, prenatal diagnoses were performed in 97, and 5 full mutation fetuses were detected. This small risk for a full mutation (only 5%) may reflect different likelihoods for premutations to expand to a full mutation in cases ascertained other than through an FXS proband (compare the two groups listed in Table 15–3). Alternatively, most of the premutations in this group may have been right at the low end of the CCG premutation range. These findings were confirmed by Berkenstadt et al.(2007) in the Israeli population where ~1 in 150 women were identified as premutation carriers. They found that the incidence of premutations in women with a family history of mental retardation was the same as that in women without such a history, but the size of the premutations in the former group was larger and thus resulted in a higher risk of expansion to full mutations.

Decisions to conduct such screening programs will depend upon funding of health care locally, and may be influenced by the frequency of carriers in the population (which is apparently higher in Israel than in other comparable populations). In a detailed economic analysis, based on the particular circumstances obtaining in The Netherlands, Wildhagen et al. (1998) determined that the cost of detecting one carrier is about US$45,000, whether testing be conducted through prenatal clinics, by preconceptual screening, or through schools. This figure contrasts with lifetime costs of care for FXS males and females of about $960,000 and $530,000, respectively. Time will have changed these amounts, but their relativity is likely similar.

A separate question is the testing of newborns for full *FMR1* mutations, which could, in principle, readily be added to an existing newborn screening program, with dried blood spots used for an assay of *FMR1* methylation. Potential benefits from early diagnosis would be to enable timely genetic counseling, ahead of the parents having subsequent children, and of earlier clinical intervention (Coffee et al., 2009).

Genetic counseling

As always, good genetic counseling depends upon accurate diagnosis. In the context of fragile XA, this means the molecular confirmation of the diagnosis of FXS and the molecular identification of carriers with good estimates of the number of repeat copies they carry. As McConkie-Rosell et al. (1999) point out, women who are carriers often have strong views about the desirability of avoiding having affected children, and want their relatives to be informed. People have different coping mechanisms to deal with the challenge of learning of their carrier status, and McConkie-Rosell et al. (2001) reproduce excerpts from a number of carriers they had interviewed, illustrating this fact. These authors have also addressed the issue of what might be suitable ages to discuss the question of the genetic risk, and actually to offer testing: each individual needs to be judged on his or her merits, and "there is no universal 'right' age" (McConkie-Rosell et al., 2002).

Consensus statements on genetic counseling for fragile X have been issued by a number of groups, and the counselor should be familiar with these (McConkie-Rosell et al., 2005; American College of Obstetricians and Gynecologists Committee on Genetics, 2006; Chitayat et al., 2008, for the Society of Obstetricians and Gynaecologists of Canada, and the Prenatal Diagnosis Committee of the Canadian College of Medical Geneticists).

The picture in FRAXE is less clear; FRAXD and FRAXF are not of practical concern.

Risks of Having an Abnormal Child

FRAXA (FMR1)

Male with Premutation.

For carrier males ("normal transmitting males") there is, according to current knowledge, virtually no risk to have a mentally impaired child due to the FRAXA gene per se. All daughters receive the *FMR1* gene in its premutation form, whatever its size in terms of triplet-repeats. It is practically unknown for a daughter to receive a full mutation. Thus, practically invariably, none would have FXS. All 46,XY sons receive their father's Y chromosome, and obviously his carrier state implies no genetic risk to them. The extremely rare instance of having a daughter with a full mutation could be mentioned, but the very low probability of this happening should be stressed (Zeesman et al., 2004).

Male with Full Mutation.

Procreation in this group is extremely rarely documented. Retrogression to a premutation in sperm dictates that daughters would be premutation carriers and (other things being equal) of normal intelligence. Sons receive the Y chromosome. There may be extremely rare exceptions to this, with a daughter receiving a full mutation (Zeesman et al., 2004).

Female with Premutation.

The risk of transmitting the FRAXA X chromosome is the typical mendelian 50%. If it is transmitted, the risk of having a child with FXS (in other words, a child with a full mutation) depends upon the size of the mother's premutation (Table 15–3), and upon the way the family came to attention. Women with "low-end" premutations of less than 60 copies of the triplet-repeat have only a very small risk to have a child with FXS; 56 copies is the smallest premutation reported to have expanded to a full mutation in one generation, from a single report (Fernandez-Carvajal et al., 2009a), as noted earlier. At the other end, for those with more than 90 copies ("high-end premutation"), the risk is in the range of 90% to 100%. The risks are substantially less in women who have been ascertained other than through a family history of FXS (Nolin et al., 2011). There is the additional concern of the neurodegenerative syndrome of FXTAS coming on in late middle age in some who inherit a premutation (Table 15–2).

Ovarian Function.

The risk for primary ovarian insufficiency/senescence (POI/S) has been noted earlier. Women with approximately 80–100 repeats are at greatest risk, with one-third experiencing premature ovarian failure (POF), although only about 4% before age 30 years. These risks are lower for those with both smaller and larger premutations. Family genetic background appears to influence the risk, and a family history of POF points to an increased likelihood (Hunter et al., 2008). POI/S, which signals a risk for subsequent POF, can be identified by measurement of anti-müllerian hormone (AMH) and follicle-stimulating hormone (FSH) (Rohr, 2008). POI/S in association with an intermediate size repeat has been reported (Streuli et al., 2009).[5] Practically speaking, it might be advisable to have children earlier rather than later in life. It is not currently

practicable to offer young women, as a routine, ovarian biopsy for cryopreservation of oöcytes for possible future use. Of course an ovum donated by a proven noncarrier sister would avoid the genetic risk.

Female with Full Mutation.

Offspring who inherit the fragile XA locus will all have full mutations. Hence, of their children with the *FMR1* mutation, all the boys will have FXS, as will about 60% of the girls. The intellectual disability in these affected females is usually less severe than in the male. A recent suggestion is that the heterozygous female child may run an additional risk, of ~5%, for X/XX mosaic Turner syndrome, possibly due to mitotic loss of the abnormal X chromosome in early embryonic development (Dobkin et al., 2009).

Gray Zone Alleles.

The possible pathogenicity of gray zones alleles has yet to be defined (see earlier). Expansion to a larger allele in a succeeding generation is rare, but recorded.

FRAXE

The fragile XE story is incomplete, and referral to a specialist in this area is necessary. For the time being, counseling should include the following caveats: *(1)* the penetrance of intellectual disability in males and females is unclear, but it may be lower than for fragile XA syndrome; *(2)* the severity of intellectual disability is less in both sexes than for fragile XA; *(3)* variation between and within families appears to be considerable; and *(4)* a notable distinction from fragile XA is that daughters of fragile XE men can have full mutations.

The phenotypic consequences of inheriting a full mutation are unclear. There is some risk of females being mildly handicapped, as yet unquantified, but probably less than for fragile XA. Some males with full mutations appear to be relatively normal, although not all have had detailed assessments. Nevertheless, we presume the risk of significant intellectual impairment in the male to be substantial, albeit less than 100%. Until the situation becomes clearer it may be best to offer prenatal diagnosis and present what is currently known to the couples involved. We await a more detailed interpretation of findings at prenatal diagnosis, but the picture is likely to be similar to FRAXA.

Prenatal Diagnosis

FRAXA (FMR1)

Preimplantation Diagnosis.

Given the high genetic risks that may apply to the female heterozygote, preimplantation genetic diagnosis (PGD) would seem, in principle, an attractive proposition. From the single (or two) biopsied blastomere, PCR of the CCG repeat region, and of a number of polymorphic markers that can define the linked haplotype, is performed (Reches et al., 2009). However, it may be difficult to obtain adequate numbers of good ova from FRAXA carriers (note the earlier comments about primary ovarian insufficiency). The prognosis for success at PGD is related to repeat size: carriers with less than 100 CCG repeat copies suffer a more impaired ovarian response and decreased fertilization rate than do those with larger premutations and full mutations (Bibi et al., 2010). Ideally only embryos with normal alleles should be transferred, since the behavior of small premutations very early in development is unclear, and it cannot be excluded that expansions to a full mutation might occur later in embryonic development.

Prenatal Diagnosis.

Chorion villus sampling (CVS) is the preferred approach. Southern analysis requires a larger DNA sample and takes more time to complete than the many PCR-based molecular diagnoses carried out for a range of inherited disorders. Times conservatively quoted for a result from Southern analysis could be 2–4 weeks (compared with 2–3 days for PCR-based results). Sufficient DNA must be extracted from the CVS sample for at least one digest (approximately 10 µg), and with sufficient additional tissue to initiate a cell culture as a source of backup DNA. Diagnosis is based on repeat length; methylation status of CVS can be misleading, and nonmethylation of CVS has been associated with methylation in fetal tissues (Castellví-bel et al., 1995). Amniocentesis is not recommended: it is done at a later gestational stage and then takes further time for cell culture to provide enough DNA. The possible outcomes of prenatal diagnoses for fragile XA are as follows:

(1) A normal male fetus (triplet-repeat copy number 6–54)
(2) A male fetus with a premutation (approximately 55–200 copies of triplet-repeat), and thus a male carrier is predicted. There is a risk, the level of which can be estimated from Table 15–2, for FXTAS.
(3) An affected male fetus with a nonmosaic full mutation, greater than 200 triplet-repeats. The FXS mental retardation syndrome is predicted. The phenotype of an affected male fetus with copy number mosaicism, that is, a mixture of full and premutations cannot be accurately predicted from CVS; most cases would be affected, at least to some extent.
(4) A normal female fetus (triplet-repeat copy number 6–54)
(5) A female fetus with a premutation (approximately 55–200 triplet-repeat copies) and thus a female carrier is predicted. She would have a risk of premature ovarian failure and FXTAS.
(6) A female fetus with a full mutation (greater than 200 triplet-repeat copies in most cells). Mental impairment, of variable degree, is predicted in at least half of full-mutation females.

FRAXE (FMR2)

There is little experience in the prenatal diagnosis of FRAXE, and expert advice should be sought.

Treatment of Fragile XA Syndrome.

The symptoms of FXS have been treated with a wide variety of behavioral and individual therapies, with varying success. Drug treatments have included stimulants and alpha-adrenergic receptor agonists, selective serotonin reuptake inhibitors (SSRIs), acetylcholine esterase inhibitors, antipsychotics for behavioral improvement, melatonin for disordered sleep, and anticonvulsants for seizures (see Chochaiya et al., 2009; D'Hulst and Kooy, 2009; Hagerman et al., 2009; and Kesler et al., 2009, for reviews). A specific rational treatment, aimed at the underlying cause of the disorder—in other words, attempting to arrest or reverse its aberrant pathophysiology—will require a good knowledge of how the lack of FMRP actually leads to neuronal dysfunction (Darnell et al., 2009). An example of this approach is to target one of the proteins normally subject to down-regulation by FMRP, and the metabotropic glutamate receptor 5 (mGluR5) is a case in point. In the absence of FMRP, an excessive amount of mGluR5 is produced, and this leads to a "long-term depression" of synaptic connectivity. Fenobam is an mGluR5-antagonist, and a trial of this drug in FXS patients is underway (Berry-Kravis et al., 2009). Another drug that may be trialed is minocycline, a tetracycline analog, which influences dendritic spine maturation in an animal model (Bilousova et al., 2009). In spite of some promise for future treatment of FXS individuals, it is improbable there will ever be a cure, but nevertheless these treatments may well have a helpful role to play in the lives of parents, carers, and the FXS individuals themselves.

Notes:

[1] There is precedent for triplet-expansion disorders having variable population frequencies. For example, the relative frequencies of Huntington disease and the somewhat similar neurodegenerative disease DRPLA (dentato-rubro-pallido-luysian atrophy) are the reverse in Japan, compared with European populations: HD is very rare in Japan, and DRPLA very rare in Europeans.

[2] There is some confusion in the way trinucleotide repeats are expressed (Sutherland and Richards, 1993). Taking the convention that the bases be written in alphabetical order from 5´ to 3´, the FRAXA repeat is CCG. Some authors "read" the opposite DNA strand, and write CGG instead (or AGG in place of CCT).

[3] A very small fraction of FXS boys look rather as though they have Prader-Willi syndrome. This effect might be a consequence of a lack of FMRP leading to an inadequate production of CYFIP (cytoplasmic interacting FMRP protein), this protein having its gene within the PWS region of chromosome 15 (Nowicki et al., 2007).

[4] The word *premutation* is well entrenched in fragile X jargon, but it betrays its etymology from when a transmitted X not displaying the fragile site was considered to be, in the 1980s, simply a state from which the true mutation might then arise. Now, as a bona fide mutation in its own right, it is stuck with this somewhat inaccurate nomenclature.

[5] An extraordinary circumstance is described in Rybak et al. (2009), concerning 32- and 26-year-old sisters, each with a 45-CCG repeat. The younger sister, having created embryos for her own future use, planned to undergo a second cycle in order to enable ovum donation for her older sister, who had already undergone menopause. The authors acknowledged an imperative "to chart a course balancing autonomy and altruism with the best interests of both recipient and donor and thereby attempt to navigate this dilemma in conformity with the ideal: first, do no harm."







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Chromosome Abnormalities and Genetic Counseling (4 ed.)

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Variant Chromosomes and Abnormalities of No Phenotypic Consequence

Chapter: Variant Chromosomes and Abnormalities of No Phenotypic Consequence
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IN THIS CHAPTER we deal with three kinds of classical chromosomal variation, having the quality in common that typically they carry no implications for abnormality in the person. First, there is the matter of normal variation. Normal chromosomes do not necessarily look exactly alike in different individuals, and some chromosomes show a remarkable degree of variation in their morphology. Obviously enough, it is crucial that the cytogeneticist distinguishes normal variation from abnormality. Generally, there is no point in reporting a particular variant to the referring practitioner or to the patient. But it is sometimes necessary to pursue the matter, with family studies, when it is not clear whether a particular finding is a normal variant or an abnormality, or when a previous report has sown a seed of doubt in the patient's mind. The study of normal variants is also a research activity in its own right.

Second, we bring together in this chapter a group somewhat flowing on from the first, comprising structural rearrangements due to the translocation of harmless material, such as heterochromatin and nucleolar organizing regions, from one chromosome to another. Third, we treat a group categorized as the "euchromatic abnormality without phenotypic effect." These are chromosomal differences that do not too comfortably accommodate the expressions "normal" and "variant," and yet "abnormality" may convey too strong a sense. *Anomaly* may be a better word, or if "abnormality" is to be used, it should retain, in the reader's mind (if not in the text hereafter), its quotation marks. These are deletions and duplications that might at first sight have been thought to be abnormalities that would be associated with some clinical defect—deletions up to 16 Mb and duplications as large as 30 Mb in size—but which are in fact observed in normal persons. Finally, in the new era of microarrays, copy number variants loom large as a new form of variation, and this demands its own chapter (which follows this one).

Biology: Chromosome Variants

If, in an individual, one chromosome of a homologous pair looks normal and the other has a different cytogenetic appearance, the pair may be said to be heteromorphic. A collection of such heteromorphisms detected with a variety of techniques is provided in Table 1 of ISCN 2009. Cytogenetic differences across the whole pool of chromosomes are referred to as variants.[1] Variants may be frequent or rare. The classical chromosome variants present a continuous spectrum, rather than a bimodal distribution. For example, considering the differing lengths of the short arms of the acrocentric chromosomes, these could be arbitrarily classified as being of approximately typical length, somewhat shorter, somewhat longer, very short, or a lot longer. They are sufficiently variable that they were exploited as markers in research (in the days before DNA markers had been well developed). Some are so rare they qualify as private variants, recorded in just a single family. We consider chromosome variation in these three areas: the centromeric heterochromatin, the acrocentric short arms, and the fragile sites.

C-Band Size, Position, and Staining Properties

C-band heterochromatin comprises, by definition, permanently inactive DNA (constitutive heterochromatin), and it is mostly located adjacent to the centromere (*C* for constitutive). It stains darkly on C-banding. The four originally described variant forms are 1qh, 9qh, 16qh, and Yqh (qh for long arm heterochromatin); the differences in size were great enough to have been detected on solid-stain chromosomes in the prebanding era. C-bands vary in size, and, for those chromosomes in which the material is centromerically placed, there is variation in position relative to the centromere (Craig-Holmes, 1977). The position of the centromere within the C-band positive heterochromatin block of the 1qh, 9qh, and 16qh may vary due to inversions of the heterochromatin. The observed frequencies vary according to the precision of staining and the criteria of the observer (Kaiser, 1988).

The commonest of these variant inversions—and indeed the commonest chromosomal variant in the human race—is the placement of 9q heterochromatin into 9p, immediately adjacent to the centromere. This high frequency presumably reflects the existence within the pericentromeric region of a number of hotspots for recombination (Starke et al., 2002). In about half of no. 9 chromosomes, a small amount (less than one-third) of the heterochromatic block is sited in the short arm. In about 10%, there is a "partial" inversion, with about one-third of the heterochromatin in the short arm, inv(9)(p11q12). In 0.6%, all the heterochromatin is in the short arm—a total inversion, inv(9)(p11q13). The wide possible range is presented in Starke et al. (2002). Possibly the most impressive 9qh variant is that recorded in Lukusa et al. (2000), in which the segment 9p11-q13 had undergone a tandem duplication. The abnormal chromosome at first sight might have offered a diagnosis for the mental defect of the woman in whom it was discovered, but her normal sister had the same chromosome, effectively exonerating it.

> Whether an "inversion variant" chromosome can influence its own disjunction is speculative. Willatt et al. (1992) observe that in 11 reported cases of the rare mosaic trisomy 9 syndrome, four occurred in the setting of maternal heterozygosity for the inv(9)(p11q12) variant and suggest a causal link; and partial trisomy 9 has been associated with a parental inv(9)(p11q12/3) (Kaiser, 1984; Stamberg and Thomas, 1986). These tiny numbers need to be seen against the background of the hundreds of thousands of inv(9) heterozygotes who have not come to cytogenetic attention for such a reason. Fortuitous coincidence, or ascertainment bias, remain a perfectly acceptable explanation.

Variation of C-band material can be demonstrated using other staining techniques, such as Q-banding, which reveal differing intensity of fluorescence. Most notably, this variation is seen in the C-bands of chromosomes 3 and 4, which range from very dull to very bright. The staining of the C-band material, after one round of replication in BrdU, varies in the pattern of lateral symmetry (Angell and Jacobs, 1978). Heteromorphic staining of the centromeric regions of all chromosomes except chromosome 8 has been demonstrated using various restriction endonucleases to treat the chromosomes prior to Giemsa staining (Babu et al., 1988). Jabs and Carpenter (1988) showed that the extra C-band material adjacent to the centromere on the short arm of a variant chromosome 6 is due to increased amounts of a chromosome-specific alphoid DNA repeat sequence.

Other unusual C-band variants are reported for chromosome 3 (Petrovic, 1988), chromosome 5 (Fineman et al., 1989), chromosome 11 (Aiello et al., 1994), chromosome 12 (De Pater et al., 2006), chromosome 18 (Pittalis et al., 1994), chromosome 20 with increased C-band material in the short arm (Fryns et al., 1988), and chromosome 20 long arm (Romain et al., 1991). The 20q variation reflects different amounts of chromosome-specific alphoid DNA. A duplication of the centromere itself is a different entity (Callen et al., 1990; Till et al., 1991).

Morphological variants of the Y chromosome are in two categories. *(1) Continuous variation* in the amount of C-band-positive heterochromatin can range from a virtual absence, in which case the Y chromosome may appear to be only about half the size of chromosome 22, to a large amount such that the chromosome is about the same size as chromosome 13. Paternal chromosome study is worthwhile to confirm that very small chromosomes are in fact variants (short Y chromosomes with breakpoints proximal to the Yq11/12 heterochromatin interface are pathogenic deletions; Salo et al., 1995). For very large ones, C-banding is adequate to confirm that the increased size is due to heterochromatin. *(2) Discontinuous variation* in the Y chromosome is expressed as a metacentric appearance, presumably due to pericentric inversion. Satellites on the end of the long arm are another variant; these are presumably due to translocation from one of the acrocentric chromosomes and have been documented to segregate in large kindreds, over centuries (Genest, 1973) (and see later in the section "NOR Translocation"). These discontinuous variants are normally without phenotypic effect, but, again, paternal chromosome study is warranted if there is any doubt.

Clinical Significance.

Many studies have purported to show that variant chromosomes involving C-band size and position are associated with congenital malformations, cancer, recurrent pregnancy loss, and infertility. There have equally been many studies that report no such association. Carothers et al. (1982) concluded, and we concur, that "reproductive fitness of carriers of heterochromatic variants of the human karyotype is normal."

Acrocentric Short Arm Morphology

The short arms of the acrocentric chromosomes show a range of morphology, reflecting variation in three components of the short arm: the centromeric heterochromatin, the satellite stalk, and the satellite material (Fig. **16–1**). These three components correlate with the three bands p11, p12, and p13. The p11 band contains several types of tandem DNA repeats, including satellites I, II, II and beta satellite; band p12 contains multiple copies of genes coding for ribosomal RNA (rRNA); and band p13 incorporates beta satellite (Piccini et al., 2001; Bandyopadhyay et al., 2001b). The nucleolus of the cell is formed by an aggregation of rRNA; thus, the stalk (band p12) is sometimes called the nucleolar organizing region (NOR). This stalk stains darkly with silver nitrate (Ag-NOR staining) on those acrocentric chromosomes that have an active NOR; and the picture varies from no uptake through marked uptake (darkness) of stain. A 3.3 kb repeat sequence, having homology to the repetitive DNA D4Z4 located at distal 4q, is found on the short arms of all the acrocentric chromosomes; and euchromatic-like sequences and transcripts exist in the short arm of chromosome 21 (Lyle et al., 2007).



Figure 16–1
Diagram of an acrocentric chromosome showing the variable components: satellite, satellite stalk, proximal short arm, and centromere.

Length and satellite size and number are distinguishing features. At one extreme, a short arm may seem to be absent; at the other extreme, it may be so long that a D-group chromosome is of C-group appearance, and a G-group chromosome has an F-group resemblance. Possibly the largest acrocentric short arm ever seen is the chromosome 15 described in Friedrich et al. (1996) in which the "short" arm was actually longer than the long arm. Molecular analysis of one chromosome 14 with an apparently absent short arm showed loss of satellite III DNA (Earle et al., 1992). Satellites vary widely in appearance: apparently absent, small or large, and single or double. Variation can occur within the one individual (Ozen et al., 1995). A meiotic exchange can lead to a parent's variant satellite appearing on another chromosome in the child (Farrell et al., 1993)—the "jumping satellites of Gimelli." The short arm of chromosome 15 stains brightly with DA/DAPI, but 18% of individuals in an English patient population, with a range of referral reasons, had extra DA-DAPI signals on other acrocentric chromosomes, 60% of them on chromosome 14; thus, an acrocentric chromosome may have its short arm originating from another acrocentric (Cockwell et al., 2007).

An apparent large acrocentric short arm can rarely mislead. Benzacken et al. (2001b) report a '14p+' chromosome discovered at late pregnancy amniocentesis (36 weeks), a fetal macrosomy having been seen on ultrasonography, and the 14p+ initially considered to be a normal variant. When the child was born, a diagnosis of Beckwith-Wiedemann syndrome (BWS) was confirmed clinically, and the infant's karyotype was reassessed. The appearance of the NORs was judged, on this occasion, to be not quite typical. Fluorescence in situ hybridization (FISH) with 11p probes convincingly showed a rcp(11;14)(p15;p13), demonstrating that the abnormal chromosome was a der(14), and that the BWS was due to a partial 11p trisomy. The parents' chromosomes were normal. De Pater et al. (2000) describe a similar circumstance, in which a prenatally diagnosed de novo 14p+ "polymorphism," which was negative on NOR staining, turned out actually to be a t(14;17)(p11;p11), and the child, who died as a neonate, thus had 17p trisomy.

Clinical Significance.

Hassold et al. (1987) could demonstrate no association of NOR variants with an increased risk for trisomic conception, a possibility that had previously been postulated. An increased risk to have a child with Down syndrome had previously been proposed for the person with a "double NOR" on an acrocentric chromosome. This was a reasonable postulate, given the in vitro cytogenetic observation of "satellite association" and imagining that a similar phenomenon could happen in vivo in meiosis, predisposing to aberrant

segregation, and that this would be more likely with larger satellites. But the actual observation is, in fact, of no such increased risk (Serra and Bova, 1990). The possibility that translocated acrocentric short arms or compound centromeres might predispose to nondisjunction and miscarriage (Cockwell et al., 2003) has not been borne out by more extensive investigations (J .C. K. Barber, personal communication, 2010)

Fragile Sites

A fragile site is a point on a chromosome that is liable, upon classical cytogenetics, to show gaps and breaks. The location of the fragile site is the same in all cells in a particular individual or family (Sutherland and Hecht, 1985). Fragile sites are classified on the basis of their frequency in the population and the conditions of tissue culture that are required to induce them.

In terms of frequency (ignoring fragile XA and fragile XE) there are three categories of fragile site (Table 16–1). *(1)* Rare sites are present only in one per several hundred individuals. *(2)* Two fragile sites are of intermediate frequency: fra(10q25), seen in 2.5% of individuals, and fra(16q22), seen in 1%–5% of individuals. *(3)* So-called common fragile sites are universal and form part of normal chromosomal architecture, and in which the underlying cause is hypoacetylation of histones in this region of the chromosome (Jiang et al., 2009). Only the rare and intermediate fragile sites are classified as chromosome variants (Fig. **16–2**). The common fragile sites, being universal, by definition do not vary; although the proportion of metaphases in which they are seen can vary, depending upon conditions of cell culture, from 0 to 20%.

Table 16–1. The Three Groups of Rare Variant Autosomal Fragile Sites

Folate-sensitive			
	1p21.3	7p11.2	11q23.3
	2p11.2	8q22.3	12q13.1
	2q11.2	9p21.1	12q24.13
	2q13	9q32	16p13.11
	2q22.3	10q23.3	19p13
	5q35	11q13.3	20p11.23
	6p23	22q13	
BrdU-inducible			
	10q25[a]	12q24.2	
Distamycin A-inducible			
	8q24.1[b]	16p12.1[b]	17p12
	11p15.1[b]	16q22.1[a]	
Unclassified			
15q13[c]			

[a] Of intermediate frequency.

[b] Recorded in Japanese populations only.

[c] Recorded in the Turkish population only

Source: From Sutherland (2003).

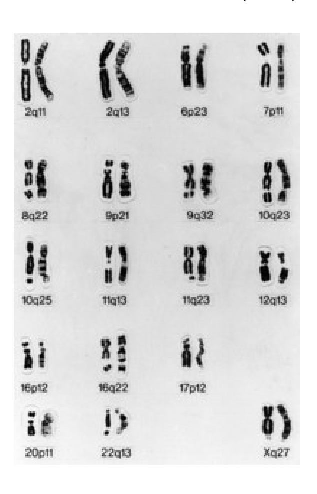









Figure 16–2
Variant autosomal fragile sites and the fragile X (FRAXA). The chromosome on the left of each pair is plain stained, and the one on the right is G banded.

FRA11B is the fragile site at 11q23.3, within the proto-oncogene *CBL2*, and which displays a molecular behavior similar to FRAXA (Jones et al., 1994). Jacobsen syndrome comprises variable deletions of 11q, and it may be that mothers of some affected children have a cytosine-cytosine-guanine (CCG) triplet expansion at FRA11B. However, in the majority of these children the deletion breakpoint is at a site elsewhere than at 11q23.3 (Penny et al., 1995; Michaelis et al., 1998).

Clinical Significance.

Fragile sites are harmless (except, of course, fragile XA and fragile XE, and, remotely possibly, FRA11B). The fragile sites of rare and intermediate frequency have been described variously as being associated with congenital malformations, sporadic chromosome abnormalities, and a predisposition to cancer. But, as noted earlier, there is no convincing evidence of any of these associations existing other than by chance (Sutherland and Baker, 2000). There is no apparent relation between autosomal fragile sites and mental retardation (Fryns and Petit, 1987). The CCG repeat at 11q23.3 might conceivably be a vulnerable point allowing de novo deletion of 11q (Jacobsen syndrome), but the evidence for this is by no means compelling. The fra(10)(q23) may lead to a del(10)(q23) being detected at prenatal diagnosis; this observation appears to be without phenotypic consequence (Zaslav et al., 2002). There is the clinical interest that one form of Seckel syndrome (p. 347) displays chromosomal breakage at fragile sites (Casper et al., 2004).

Fragile sites that have been characterized at the DNA level are all in genes that are silenced by hypermethylation when the fragile site is present. Theoretically, homozygosity might have been expected to result in an abnormal phenotype, depending upon the nature of the gene involved; but in fact no such homozygosity has been recorded. Homozygosity for some folate-insensitive fragile sites (10q25, 16q22, 17p12) is described; the individuals studied had no consistent phenotypic abnormality, possibly because these fragile sites are located in highly repetitive DNA areas devoid of genes.

Y Heterochromatin Translocations

In the sense that they cause no phenotypic abnormality, the so-called t(Y;15) and t(Y;22) translocations, in which the C-band material from the long arm of the Y has been translocated onto the short arm of the acrocentric, can be regarded as examples of normal heteromorphism (Friedrich and Nielsen, 1972; Cohen et al., 1981; Neumann et al., 1992). Once formed, these variant chromosomes are stable. In one reported family, a girl with Prader-Willi syndrome had a de novo 15q11-q13 microdeletion on a familial t(Y;15)(q12;p11) chromosome. Her father, brother, uncle, and two aunts also carried the intact t(Y;15) (Eliez et al., 1997). Ascertainment bias is the more likely explanation of such coincidences.

A segment of Y chromosome heterochromatin can be insertionally translocated into another chromosome, and this may be without any phenotypic effect. Ashton-Prolla et al. (1997) report detecting such an anomaly at prenatal diagnosis, with the karyotype only interpretable after the father had been studied. He had a segment from Yq12 inserted into chromosome 11 at band 11q24: 46,XY,der(11)ins(11;Y)(q24;q12q12). He had inherited this ins(11;Y) from his mother, and her normality, and subsequently his infant daughter's normality, attested to the innocuousness of this variant chromosome. A similar case is described in Spak et al. (1989), of a harmless C-band positive insertion at 11q23.2; the origin of the heterochromatin could have been an autosome or the Y chromosome.

Clinical Significance.

These Y heterochromatin translocations are to be regarded as variants without phenotypic consequence.

NOR Translocation

The NOR can be translocated to another chromosome, usually to a terminal region; the nomenclature of *ps* or *qs* denotes a satellite (that is, a NOR) at the tip of the arm in question. Willatt et al. (2001a) document examples of this phenomenon, either as terminal or as interstitial NORs, in chromosomes 1, 2, 4 (several cases), 6, 7, 8, 10, 12, 17, and 22, and Faivre et al. (1999) discuss the recorded cases identified at prenatal diagnosis, noting that in only one of 13 was there an otherwise unexplained abnormal phenotype, and acknowledging that this could have been a coincidental association. The anomalous chromosome can arise somatically, as Storto et al. (1999) show in a father with 46,XY/46,XY,10qs mosaicism, whose (normal) child had been diagnosed with the 10qs at amniocentesis.

Wilkinson and Crolla (1993) showed, in the Yqs chromosomes segregating in three families, that the NOR on the Y in each had arisen from a 15p to Yq translocation, as did two of the four cases of Kühl et al. (2001). These Yqs chromosomes have lost their pseudoautosomal region-2, but this is of no discernible clinical consequence. Reddy and Sulcova (1998b) undertook detailed molecular dissections of three NOR translocations: a chromosome 21 into the short arm of which segments of chromosome 15 beta-satellite elements were inserted, making it “tricentric”; a chromosome 7 with acrocentric beta-satellite DNA inserted at 7p13; and a pseudodicentric chromosome 2 with beta-satellite inserted at the tip of 2q.

Notwithstanding the typical circumstance of harmlessness, the NOR translocation may, in rare instances, and if there is a deletion or disruption at the point of attachment, be pathogenic. Chen et al. (2000) report their experience with a “4ps” in a mother and Wolf-Hirschhorn syndrome in her child, but in fact this reflected unbalanced segregation from a reciprocal translocation t(4;15)(p16;p11.1). Their second case of an Xqs chromosome is more telling, in which a 46,X,Xqs mother had an abnormal 46,Xqs,Y son. They proved the point that there was a distal Xq deletion, and so the abnormal chromosome might more accurately be described as Xpter→Xq28::sat, rather than Xpter→Xqter::sat. A notable example of a possibly pathogenic insertional NOR translocation is that described in Tamagaki et al. (2000) (and see p. [link]), in which an NOR inserted interstitially at Xq11.2 was segregating with a spastic paraplegia in the males. The cosegregation could be coincidental, but equally might reflect disruption of a causative X-borne locus due to the NOR insertion.

Clinical Significance.

In general, these satellited chromosomes are to be regarded as harmless heteromorphisms. Faivre et al. (2000a) reasonably comment, in their study of a familial 4qs ascertained through a child with a cerebellar ataxia, that “genetic counseling should be reassuring” in the setting of an NOR translocation identified at prenatal diagnosis. We note earlier rare exceptions to this rule, in which the translocated NOR may have caused genomic disruption at the site of translocation. The lesson from these cases is that any satellited chromosome deserves detailed study (especially an X chromosome NOR not known to be carried by a phenotypically normal male in the family), while retaining the perspective that, very probably, the conclusion will be that it truly is a harmless variant. Subtelomere FISH or microarray analysis may be reassuring to a family with a de novo telomere-NOR translocation, by demonstrating that no material of consequence is missing.

Chromosome Anomalies

Euchromatic Abnormalities of No, or Uncertain, Phenotypic Consequence

The G-band pattern is generally constant, and the relative sizes of G-bands are similar between the karyotypes of any members of the human race. The fact of being a diploid species is useful, enabling one homolog to be compared with the other in any metaphase. Most of the banding pattern variation observed by the cytogeneticist is artifactual. The level of resolution, which is a function of the degree of compaction of the chromatin, will determine the number of bands seen in any metaphase, and even within a metaphase the number of bands on homologous chromosomes may vary (“homolog asynchrony”). Two major categories, at the cytogenetic level, may be considered (Barber, 2005) (the category due to molecular-level copy number variation is treated in the next chapter):

- Euchromatic variants due to constitutional cytogenetic amplification

- Euchromatic deletions and duplications with no, or uncertain, phenotypic effect

A distinction between one of these anomalies and a pathogenic cytogenetic abnormality may not always be easy. It is generally considered that if, in a family study, all the individuals (with the usually allowable exception of the index case) having the unusual chromosome are phenotypically normal, then the chromosome really is a euchromatic abnormality of no phenotypic consequence. And yet, some chromosome anomalies, judged to be harmless by this criterion, might have a euchromatic deletion or duplication that is actually of similar extent to some of those which are undoubtedly pathogenic.

How can there be such a difference in outcome: a normal or an abnormal individual? What matters is the nature of the loci in the segment of interest. Is this a region of low gene content? Harmless deletions frequently involve G-band dark euchromatin with relatively low gene density (Barber et al., 2005; Daniel et al., 2007), but at least one harmless duplication of a region of moderate gene density has been described (Barber et al., 2007). Are the genes dosage sensitive? If a gene, or a set of genes, function equally well at a 50% as at a 100% level, a deletion on one chromosome will be without any effect upon phenotypic expression and could be thought of as being “haplo sufficient.” Are the genes part of a family of genes or a network of genes of related function? If so, it is possible that other genes in the same family or network may compensate for the loss of one of their members (Barber et al., 2005, 2006). Rarely, a parent-of-origin effect is invoked (Temple et al., 1996). Finally, the interpretation of phenotypic normality needs to made with some confidence. It could be that a deletion or duplication in a gene-sparse region might lead to only a very subtle compromise of form and function, just within the range of normality; but more detailed clinical assessment could show that the chromosome abnormality is actually, if subtly, pathogenic (Lopez-Exposito et al., 2008).

Euchromatic Variants

In seven chromosome regions, an additional band or bands may infrequently be discerned. These bands reflect changes of known copy number variable regions, as listed in the Database of Genome Variants (http://projects.tcag.ca/variation). When the copy number is high enough, they become visible in the light microscope and may be described as “euchromatic variants”; Barber (2008a) also refers to “chromosomal copy number variants.” An additional large tract of segmentally duplicated euchromatin may have a similar appearance (Willatt et al., 2007). The regions of interest comprise 4p16, 8p23.1, 9p12, 9q12, 9q13, 15q11.2, and 16p11.2. With few exceptions, no major clinical significance attaches to the discovery of any of these variants (Barber, 2005). We provide brief commentaries on each.

Band 4p16.1.

A cytogenetically visible amplification of 4p16.1 cosegregated with ear and eye defects in a large family reported by Balikova et al. (2008). Five tandem copies of a 750 kb amplicon were present on the variant chromosome. The amplicon contained olfactory receptor, defensin, and *DUB* gene family members, which are known, individually, to be copy number variable. The amplicon in this family may have been the basis of the defects inherited in a mendelian fashion (in which case, euchromatic “variant” would be a misnomer) but, alternatively, the variant may simply have been cosegregating with a closely linked mutation.

Band 8p23.1.

A number of families with euchromatic variants of 8p23.1 and a normal phenotype have been described (Barber et al., 1998, Barber, 2005). The anomalous G-light band appears larger with a fine G-dark band in the middle of the enlarged band. The additional material contains multiple copies of a 260 kb amplicon of olfactory receptor and defensin genes (Hollox et al., 2003). Variant carriers have a total of 8–12 copies of this amplicon compared with the 2–7 copies found in the general population. Copy number variation of this region is normally benign, except for a predisposition to psoriasis with increasing copy number (Hollox et al., 2008). Predisposition to Crohn's disease from reduced (Fellermann et al., 2006) or increased copy number (Bentley et al., 2010) has not been confirmed in larger series of patients (Aldhous et al., 2010). Distinction is to be made from the known pathogenic 8p23.1 duplication, which is cytogenetically indistinguishable from the euchromatic variant and which can arise de novo, or be transmitted from parent to child (Barber et al., 2008; Barber et al., 2010).

Pericentromeric Chromosome 9.

There are at least four kinds of euchromatic variants of chromosome 9 which reflect the existence of pericentromeric segmental duplications that are themselves copy-number variable (Redon et al., 2006). In the short arm, an extra euchromatic band is seen between 9p11 and 9p13. This may be due either to multiple copies of a ∼1 Mb amplicon (Lecce et al., 2006) or to extra copies of large tracts of segmentally duplicated material (Willatt et al., 2007). In the long arm, extra bands may exist within the major block of long arm heterochromatin in 9q12/9qh, or in the proximal long arm between 9q13 and 9q21.2; deletion of 9q13 to 9q21.12 (and perhaps 9q21.2) may also be observed (Joseph-George et al., 2011). Duplication, triplication, or deletion of segmentally duplicated material accounts for the extra or missing bands. No clear phenotypic effects have been demonstrated and the suggestion of an association with recurrent pregnancy loss remains seems unlikely (Dundar et al., 2008; Barber, 2010).

Band 15q11.2.

The importance of these 15q11.2 euchromatic variants lies in the need to distinguish them from the pathogenic 15q11.2–15q13 duplications (and triplications) of the Prader-Willi/Angelman critical region (PWACR) (p. 329), which can look very similar under the light microscope (Barber, 2005). The extra band (or bands) reflects amplification of a cassette of pseudogenes derived from the neurofibromatosis type 1 (*NF1*), immunoglobulin heavy chain D (*IgH D*), gamma-amino-butyric acid receptor 5 (*GABRA5*), and B-cell lymphoma genes (*BCL8*) (Fantes et al., 2002). These are located between the centromere and the most proximal breakpoint (BP1) of the PWACR. Copy number of the *NF1* and *GABRA5* pseudogenes is 1–4 in normal controls, and 5–10 in variants.

Band 16p11.2.

A number of families are known with an apparent duplication of band 16p11.2 (Barber, 2005; Lopez Pajares et al., 2006). The euchromatic variant, which lies practically adjacent to the pericentromeric heterochromatin, can be extensive, almost doubling the overall size of the short arm. The extra band is due to amplification of a pseudogene cassette that is derived from immunoglobulin heavy chain D (*IgH D*), creatine transporter (*SLC6A8*), and adrenoleukodystrophy (*ALD*) genes. Copy number in controls has been estimated as 4 and between 10 and 12 in variant carriers. Distinction is to be made from pathogenic duplications of 16p11.2 (p. 330).

Band 16q11.2.

An additional G-light band within the major 16q11.2/16qh block of heterochromatin may result from an inverted duplication of the centromere (Jalal et al., 1990). This needs to distinguished from pathogenic duplications of 16q12.1-q13 (Barber et al., 2006), which can appear to be inserted into the 16q11.2/16qh block.

Euchromatic Duplications and Deletions with No, or Uncertain, Phenotype Effect

Thinking purely in mechanical and structural terms, these duplications and deletions of little or no phenotypic effect may be no different, in terms of structure, from a pathogenic abnormality of similar size. That is, a similar process has led to the addition or removal of a segment of chromatin. As discussed earlier, it is likely that the nature of the segment in question is what determines harmlessness or pathogenicity: put simply, a gain or a loss of genes that does or does not matter. Or, if it does matter, it may matter very little, and the effect of the gain or loss may be so mild that the phenotype is only subtly influenced, which thus remains well within the range of normality.

Quite a number of these cytogenetically visible duplications and deletions have been reported (and several more unpublished examples are likely known in a number of cytogenetics laboratories). Barber (2005) has undertaken a review, and Table 16–2 sets out the harmless deletions and duplications that have been recorded. Most of these unusual chromosomes had been detected in normal persons, and the same then in normal relatives; prenatal diagnosis is also a frequent route of ascertainment. In other cases (Table 16–3), the chromosome was first discovered in an abnormal individual, but then the same chromosome was identified in other phenotypically normal relatives. This observation of normality in other family members would typically allow the presumption of the chromosome being simply a variant, although one should be careful about

extrapolation to different families in which the same unbalanced chromosome abnormality is observed.

Table 16–2. Euchromatic Duplications, Deletions, and “Variants” without Phenotypic Effect, as Inferred from the Observation of Transmission from Phenotypically Normal Parent to Normal Child

CHROMOSOME	del	dup	VARIANT
1		p21-p31	
		q31.1-q32	
2	p12-p12		
	q13-q14.1		
3	p25.3-pter	q28-q29	
4	q34.1-q34.3	p16.1p16.1	
5	p14-p14		
6	p25-pter		
	q22.31-q23.1		
7	p22.3-pter		
8	p23.1/2-pter	p23.1-p23.3	p23.1
	q24.13-q24.22		
9	p21.2-p22.1	p11.2-p13.1	p12
			q12
			q13-q21.2
10	q11.2q21.2	p13-p14	
11	p12-p12	q14.1q23.1	
12		q21.31q22	
13	q14.3-q21.33	q13-q14.3	
		q14-q21	
15			q11.2
16	q13q22		p11.2
18	p11.31-pter	p11.2-pter	
		q11.2q12.2	
22	p11.21-pter		

Note: The estimated sizes of the deletions and duplications range from 4.2–16.0 Mb (del) and 3.4–31.3 Mb (dup).

Source: From Barber (2005) with additional material from the Chromosome Anomaly Collection Web site. Updated information is posted in the “What's New” section at http://www.ngrl.org.uk/Wessex/collection/index.htm

Table 16–3. Euchromatic Deletions or Duplications in Which the Child Was Abnormal But the Parent Normal

CHROMOSOME	del	dup
1		q11-q22
		q42.11-q42.12
2	q13-q13	
	q14.1-q14.2	
3	p25.3-pter	
		p11.2-p12.3
		q25-q25
4		q31.3-q33
		q35.1-q35.2
5	p15.3-pter	q15-q21
	p14.1-p14.3	
6		q23.3-q24.2*
7	p22-pter	
8	p23.1-p23.2	p23.2-p23.2
10	q11.2q11.23	
11	q14.3-q22.1	
13	q14-q14**	q21.1-q21.32
14		q24.3-q31
15		q11-q13
		q11-q12
16		q12.1-q12.1
18		q21.31-q22.2
21	q21.1-pter	

Notes: The estimated sizes range from 3.6–10.0 Mb (del) and 2.0–16.3 Mb (dup). In several of these cases, the abnormality in the child is likely to have been coincidental, and the chromosomal abnormality was, of itself, without phenotypic effect. In some, however, a causal link must be presumed, such as the footnoted dup 6q24 and del 13q14, albeit in these two requiring the agency of another factor (imprinting effect; mutation on other homolog).

* Child with transient neonatal diabetes, presumed to have been a consequence of paternal disomy for this segment (Temple et al., 1996).

** Child had retinoblastoma, presumably due to a second hit at the *RB* locus at 13q14 on the other chromosome 13 in a retinal cell (Cowell et al., 1988).

Source: From Barber (2005), with additional material from the Chromosome Anomaly Collection Web site. Updated information is accessible at http://www.ngrl.org.uk/Wessex/collection/index.htm

In a few families, the child's abnormality may have been the direct result of the “chromosomal variant,” the parent's normality notwithstanding, and a number of explanations can be proposed. Variable penetrance and expressivity, more traditionally invoked in mendelian genetics, are gaining a broader acceptance in cytogenetics, with the 1q21.1 microdeletion in thrombocytopenia absent radius (TAR) syndrome a notable example; Klopocki et al. (2007) propose an unidentified “modifier” of expression to explain their finding that, in 75% of families, the clinically normal parents will have the same 1q21.1 microdeletion (see also p. 311). A similar explanation may apply to distal 3p deletions that may or may not lead to a phenotype (Barber, 2008b).

The distinction between a euchromatic abnormality of no phenotypic consequence and one of only slight consequence may be a very subtle one. Consider the case of a girl described in Bonaglia et al. (2002) who had a karyotype ordered on fairly slender grounds at age 18 months (she was in hospital at the time for an ear infection), but as a 6½ year old had only the most minor signs (one ear ½ cm longer than the other, and crowded teeth, for example), and IQ testing at 5½ years had shown an average intelligence. She had a de novo dup(9)(p22→p13), proven to have arisen following an asymmetric sister chromatid exchange at paternal meiosis. Would she have been slightly different in appearance, her dentition less imperfect, and her IQ a little higher, if she had had an apparently normal karyotype 46,XX? Or would she have been just the same, and the dup(9p) was truly a euchromatic abnormality of no phenotypic consequence? Since dental crowding is seen in the classical 9p duplication syndrome, perhaps this aspect truly is due to the karyotypic imbalance.

To recapitulate: at the fundamental level of cytogenetic mechanism, at least some of the abnormalities with and some without phenotypic effect may have arisen in essentially the same way, and the difference depends upon whether the genes duplicated or deleted were dosage sensitive (and recognition naturally depends upon whether their effects are observable at clinical examination).

Subtelomeric Deletions and Duplications

Some “deletions” and “duplications” identified on subtelomeric FISH probing may well be true findings, but being without effect upon the phenotype (Riegel et al., 2001). Ballif et al. (2000a) record, for example, the following (sub)telomeric variants: ish add(1)(13qtel+) using PAC probe 163C9; ish del(2)(qter) with PAC 1011O17 and P1 210E14; ish del (9)(pter) with PAC 43N6; and ish del(X)(pter) with cosmid CY29. The harmlessness (in all probability) of these variants was demonstrated, as per usual, by showing the same thing in a parent. Many more such variants have been discovered, as subtelomeric analysis has become routine using FISH and/or multiplex ligation-dependent amplification (MLPA), and more surely will be, as the use of microarray-CGH becomes more widespread (Adeyinka et al., 2005; Ravnan et al., 2006; Balikova et al., 2007; Martin et al., 2007). The normal phenotype in many of these euchromatic subtelomeric deletions and duplications may possibly be explained by the degree to which they are composed of tandem duplications (Linardopoulou et al., 2005). Informed interpretation of these findings will require of the molecular cytogeneticist a ready ability to access information in the literature and on genome browsers, and an awareness of the best probes to use in order to distinguish between variants and true pathogenic abnormalities.

Details of Meiotic Behavior.

Variant chromosomes, being normal chromosomes, behave normally at meiosis; and other things being equal, 1:1 segregation occurs. Hence, any individual with a variant chromosome transmits it to, on average, half of his or her offspring. In the specific case of the inv(9)(p11q13), an essentially 1:1 ratio was confirmed in a sperm study (Colls et al., 1997). Similarly, most simple duplications and deletions are directly transmitted, intact, in a 1:1 ratio.

Very rarely, an apparently nonpathogenic duplication in a parent can undergo replication that leads to a pathogenic triplication in a child, and Yobb et al. (2005) and López-Expósito et al. (2008) provide examples with respect to parental duplications of a 3 Mb segment in 22q11.2, and a 12 Mb segment in 13q21.1-q21.33, respectively.

Genetic Counseling

Reproductive Advice

A person carrying a variant chromosome has, practically by definition, no increased risk for having abnormal offspring, pregnancy loss, or any other reproductive problem. Some see it as at best pointless and at worst counterproductive even to mention to the individual that a variant chromosome has been found; others feel obliged to pass on the observation. If it is discussed, it must be made clear that it is a normal finding: perhaps interesting, but of no practical importance. (Some patients may be intrigued to learn that they are of interest to genetic researchers.) For the size variants (C-band and NOR), the point can simply be made that some chromosomes come in short, medium, and long forms, and where a chromosome happens to fit in this continuum is without significance.

There is considerable potential for iatrogenic anxiety, whereas in reality the biology of the supposed anomaly has no pathogenic implication. The counselor may thoroughly understand the presumed harmlessness of a variant chromosome, but the person in whose family it has been discovered may react “nonscientifically.” To put a stark setting, the worst possible response might be for a couple to choose to terminate a pregnancy because of an overinterpreted variant chromosome, as has actually happened with the 16p11.2 euchromatic variant (López Pajares et al., 2006). “*Primum non nocere*”: first do no harm.

Parent with Same Variant as Abnormal Child

From time to time, children with delayed neurodevelopmental progress with or without minor dysmorphic features are identified as having a subtle deletion or duplication; and in most, of course, the karyotypic abnormality will have been the cause of the phenotypic defect. Occasionally, parental studies will come up with the surprising result that one parent has the same karyotype and a reinterpretation of probable “harmless variant” may be made. The counselor will need to make sure that this reinterpretation is well understood and that the label of “chromosome abnormality” does not attach to the child and stifle other clinical investigation. Equally, the evolving understanding that some “variants” might actually be truly pathogenic, but of low penetrance (with most carriers unaffected), will oblige a nuanced approach. The counselor will need to keep abreast of the literature regarding the significance of these inherited genomic changes.

Cancer.

Persons who have variant fragile sites (we all have the common fragile sites) may be advised, if the question is raised, that there is no evidence of an increased risk for cancer (Simmers et al., 1987; Sutherland, 1988; Sutherland and Simmers, 1988). There is emerging evidence that some of the common fragile sites have within them tumor suppressor genes that can be disrupted in some cancers (Arlt et al., 2003). But there is no indication of increased risk for an individual in whom a particular common fragile site may be prominent (in terms of the proportions of metaphases in which it is seen).

Notes:

[1] The word *polymorphism* is also used as a cytogenetic colloquialism in this context, but, to be precise, it is an inaccurate usage. The genetic term *polymorphism* refers to the presence of an uncommon allele that has a frequency of >1% in the population, and although some chromosome variants may be more common, each one (e.g., 9qh+) is not necessarily homogeneous but may comprise a collection of many “alleles” at this locus. Darwin had this comment, from his *Origin of Species*: “Variations neither useful nor injurious would not be affected by natural selection, and would be left a fluctuating element, as perhaps we see in the species called polymorphic.”



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Copy Number Changes : Benign Variants And Changes Of Unclear Clinical Relevance

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THE MICROSCOPE has long been the classical instrument of the cytogeneticist, and large-scale variation in the human genome has been known since the early days of the discipline. As identified by the examination of banded chromosomes, variation can be normal or abnormal. Normal variation includes rearrangements or visible differences that are seen in the general population, and which are without clinical significance. These include the common pericentric inversion of chromosome 9, and the morphological and staining differences between homologs of the acrocentric short arms, and of the pericentromeric heterochromatin of certain chromosomes, and these have been discussed in the preceding chapter. Abnormal variation includes balanced rearrangements such as inversions, reciprocal translocations, and Robertsonian translocations, and unbalanced rearrangements such as derivative chromosomes and supernumerary marker chromosomes. Thus, the traditional study of human chromosomes with banding techniques has identified large-scale genomic changes, typically referred to as chromosome abnormalities, for over 40 years.

The new instrument of the twenty-first century cytogeneticist is the microarray (as described in detail in Chapter 2). This new approach has revealed a new layer of variation: genomic variation ranging from a few to several million base pairs of DNA, in clinically normal individuals. The interpretation of such variation can be that these are normal, abnormal, or of unclear clinical significance. This chapter will explore normal genomic variation and discuss when the lines blur between normal and clinically abnormal, as well as what tools are useful for distinguishing this variation.

Microarray Analysis

Chromosome microscopy can identify deletions and rearrangements that involve chromosomal segments greater than 5–10 Mb of DNA and that typically result in an obvious change in banding pattern. Alterations smaller than 5 Mb may require molecular methods to visualize. The microscope-based method of fluorescence in situ hybridization (FISH) requires knowledge of where to go “FISHing” to identify a chromosome abnormality. With the advent of microarrays, the entire genome can be scanned for alterations in a single hybridization. Recently, microarray-based chromosome analysis has overtaken traditional chromosome analysis and FISH as the laboratory method of choice for identifying chromosome abnormalities. However, this widespread use of microarray analysis has led to the identification of alterations not previously recognized. It is the job of the cytogeneticist to interpret these alterations and to provide helpful information to the referring physician and genetic counselor. It is then the job of the genetic counselor to convey and interpret the information to the family.

Microarray analysis is not a stand-alone test in the diagnostic laboratory, because it cannot elucidate the molecular mechanism that caused the DNA gain or loss, and it cannot reveal the type of rearrangement or the resulting abnormal chromosome structure. The identification of the precise chromosome rearrangement is necessary for proper genetic counseling. For example, the identification of an unbalanced translocation in a child means that one or the other parent could be a carrier of a balanced translocation. As discussed at length in this book, balanced translocation carriers may be at substantial risk for having additional children with the unbalanced form of the rearrangement. Therefore, because the information from a microarray is incomplete in regard to the type of rearrangement, microarray results should be confirmed by FISH to visualize the structure of the abnormal chromosome. For some smaller alterations, FISH analysis may not be possible because the size of the FISH probe is larger than the chromosomal alteration. In these instances, quantitative fluorescent polymerase chain reaction (QF-PCR) or multiplex ligation-dependent probe amplification (MLPA) (Chapter 2) can be used to confirm the gain or loss; although neither of these methods uncovers the nature of the rearranged chromosome. It is true to say that microarray analysis has completely changed the face of cytogenetics and has enabled the detection of chromosome abnormalities at an unprecedented resolution, and in a considerably greater fraction of patients.

Definitions

Since the identification of the first genomic alterations in normal populations, the literature has been inundated with new terms and attempts at nomenclature, in order to describe the gains and losses observed from microarray analysis. It may be helpful to define the terms as will be used in this chapter.

Copy Number Variation.

Because cytogeneticists typically refer to “variation” as meaning “normal,” the word *variation*, or the expression “copy number variation” (CNV), will be used to indicate a benign change in the genome that does not (to the best of *present* knowledge[1]) have any phenotypic effect. It is to be seen as a normal observation. (In these early years of the twenty-first century, the broader concept of the CNV is generating a very large literature, well beyond the narrow confines of its relevance to clinical cytogenetics, and we leave this to the interested reader to pursue.)

Copy Number Change.

On first discovery, the significance of a difference in copy number may not be understood. In general, and if we imagine a “standard genome,” most regions of the genome are

represented in two copies, one on each chromosome, albeit that there are some instances of four or more copies of a gene, usually in tandem or near to the “original” copy, with two or more copies on each chromosome, in normal individuals. Thus, copy number change (CNC) refers to a change that is not yet determined to be benign, or clinically relevant with phenotypic consequence. It is simply a gain or loss in which the inheritance may or may not be known. A copy number alteration (CNA) that has clear phenotypic consequence to the individual is not normal: it is a chromosome abnormality and does not have a place in this chapter.

Normal Results by Microarray.

Such a report indicates that there is no DNA gain or loss of clinical relevance to the phenotype of the individual. The individual may show one or more CNVs, but they are not believed to be relevant to the phenotype. This normal result does not exclude a mutation in a gene that is not detectable by microarray analysis, and neither are balanced rearrangements such as reciprocal translocations and inversions excluded, these not being detectable by microarray.

Abnormal Results by Microarray.

Such a report indicates that there is a DNA gain or loss that is presumed to be causative of the patient's phenotype. This determination is usually based on the laboratory's experience with other such patients, with one of the evolving CNV databases, and the available literature on a particular alteration. This determination cannot always be based on size of the alteration or genetic content alone, as some fairly large CNVs (1–2 Mb) are being recognized.

Incidental Discovery of Whole Gene Duplication or Deletion.

A microarray analysis that interrogates the whole genome may reveal a missing or an extra copy of a gene, quite unrelated to the clinical reason for the test having been ordered. Many genes are not dosage-sensitive in the duplicated state, and for example the incidental discovery of a duplication of the *KAL1* gene on the X chromosome would not have any clinical implication (whereas mutation or deletion leads to Kallmann syndrome). However, duplication of *PMP22* is certainly pathogenic, and it is the basis of one form of Charcot-Marie-Tooth disease (p. 330); and we refer on p. 16 to the incidental discovery of a duplication that has been associated with an ataxic syndrome. Deletion of a whole gene that is dosage-sensitive or subject to haplo-insufficiency would be pathogenic in the context of dominantly inherited disease; for example, a few cases of the cancer-predisposition syndrome hereditary nonpolyposis colon cancer are due to complete deletions of the *MLH1* gene, or of the *MSH2* gene. Deletion of one copy of a recessive gene (in the presence of a normal allele on the other homolog) would merely lead to recognition of the carrier state in that individual. We have seen, for example, single-copy deletion of the *NPHP1* locus at 2q13 in a number of samples referred from Québec, this gene the basis of, remarkably, both a renal disease (juvenile nephronophthisis) and a cerebellar disorder (Joubert syndrome). Disease would of course result if both copies were deleted, or if the other chromosome happened to carry a typical mendelian mutation. The ethical issues raised by these discoveries are discussed on p. 15.

Results of Unclear Clinical Significance.

Rather frequently the microarray analysis reveals a DNA copy number gain or loss, but it is unclear whether this alteration is responsible for the patient's phenotype. The laboratory or physician may be unable to assign a clinical relevance to the alteration because it has never been seen before in that laboratory and does not appear in the available literature. Parental testing can be helpful in some cases, as discussed in more detail later. Briefly, if neither parent carries the alteration and paternity is known to be correct, then the gain or loss is de novo and could well be contributing to the phenotype; but equally one must exercise caution, since de novo benign CNVs may also arise (Sharp, 2009). But if one parent carries the alteration and clinical information is not available, or if the parent is clinically abnormal but not necessarily with the same symptoms as the child, the ability to interpret the DNA change becomes more difficult. More specific examples are given later regarding recent evidence that some alterations appear more frequently in the patient populations being studied for a variety of medical issues than in the general, normal population. However, most often in the abnormal patient population, these alterations are inherited from a clinically normal parent. These particular alterations may confer a predisposition to medical problems (a “susceptibility locus”) or represent a modifier to some other, unknown, alteration. It is unclear how many of these types of alterations exist in the population, and this becomes very challenging when encountered in the prenatal setting. As Friedman et al. (2009) rather soberly comment, “it seems likely … that no perfect array genomic hybridization platform for detection of pathogenic CNVs may ever exist and that effective clinical interpretation of these studies will continue to require considerable skill and experience.”

Normal Population Variants

CNVs, as defined in this chapter, are gains or losses in the genome that are found among individuals in the general, normal population. The frequencies of each may vary depending on the ethnic background of the individuals tested. The sizes can also vary, from less than 1 kb to a few megabases. The finding of a genomic gain or loss in a child with an abnormal phenotype may require the investigation of parental samples to identify whether the alteration was de novo or inherited. The finding of an inherited alteration lessens the likelihood of a causative relationship between the abnormal phenotype and the genomic change, although causation cannot be completely excluded in all cases because of variability in penetrance and expressivity[2] of some microdeletions and microduplications (Mencarelli et al., 2008; Buysse et al., 2009). The finding of a de novo change is usually interpreted as causative, but the gene content of this genomic segment should be considered in the context of the patient's phenotype.

In the first studies reported of normal individuals, an average of about 12 CNVs per individual was identified (Iafrate et al., 2004; Sebat et al., 2004). Many studies have evaluated the usefulness of microarrays in the detection of chromosome abnormalities in children with disabilities. CNVs and DNA copy number changes of unclear clinical relevance have often been noted, and Table 17–1 summarizes the earliest of these studies in which 100 or more individuals were tested.

Table 17–1. Detection Rates of Copy Number Variations (CNVs) in Studies of 100 or More Individuals

NO. OF INDIVIDUALS	MICROARRAY TYPE, RESOLUTION	INHERITED OR BENIGN CNV RATE	REFERENCE
270	Affymetrix GeneChip 500K and BAC whole genome tiling array	12%	Redon et al., 2006
100	BAC, 100 kb	258/78 normal parents[a]	de Vries et al., 2005
140	BAC, 1 Mb	6%	Menten et al., 2006
1500	SignatureChip, BAC, targeted	2%	Shaffer et al., 2006
100	Affymetrix GeneChip, 100K	3125/100 children[b]	Friedman et al., 2006
104	Affymetrix GeneChip, 100K	>100%	Hoyer et al., 2007
2444	BAC, targeted	9%	Lu et al., 2007
8789	SignatureChip, BAC, targeted	1%	Shaffer et al., 2007
373	SignatureChip, BAC, targeted	4%	Baris et al., 2007
207	Oligonucleotide, 500 kb	2%	Baldwin et al., 2008
1151	Spectral Genomics, BAC	4%	Pickering et al., 2008

[a] 258 alterations were found in 78 of the tested clinically normal parents of 100 children with idiopathic intellectual disability.

[b] 3125 CNVs that “probably represent normal polymorphisms” were identified in 100 children with idiopathic intellectual disability.

The differences in reported frequencies between studies may be due to the kind of array used (BAC, high-density oligonucleotide array, or SNP array) and reporting criteria. Reporting criteria vary widely, with some laboratories relying on internal patient databases, external population databases, and gene content, while other laboratories use an arbitrary cutoff based on size. Because the inclusion criteria of the study subjects and the microarray resolutions differ, it is difficult to combine all studies to find an overall rate of CNVs. Meta-analysis of reported data showed that more than 20% of the genome was interpreted as a CNV (Cooper et al., 2007; Miller et al., 2010).

The array platforms that utilize high-density coverage of the entire genome, irrespective of known CNVs or known clinically relevant regions, have shown up to 31 CNVs per individual studied (Friedman et al., 2006). Every individual showed variation, and most showed many different genomic regions displaying gains and losses. This is in sharp contrast to the targeted microarrays, which show as little as 1%–2% CNVs (Shaffer et al., 2006b, 2007a). The design of the microarray, by avoiding known polymorphic CNVs, can minimize the number of cases requiring parental samples to interpret a child's result, and it lessens the chance of misinterpreting a benign change as a pathogenic alteration and vice versa.

Results of Unclear Clinical Significance

There are many circumstances in which findings may be of unclear clinical relevance, with respect to an alteration that has not been reported before. If de novo, the alteration more likely contributes to the phenotype. If the alteration is inherited, it may remain unclear because clinical information on the parents is not available, the parent may be clinically abnormal, or the CNC is known to be associated with an abnormal phenotype in some individuals, but not all carriers. Thus, the paradigm for distinguishing pathogenic from benign genomic changes has shifted, and one cannot assume that all DNA changes are fully penetrant. Even de novo changes may not all be pathogenic. Although the mutation rate is unknown, de novo, benign CNV formation cannot be excluded (Sharp, 2009).

Some studies have suggested that there is a correlation between the size of the alteration and the probability of its being de novo (de Vries et al., 2005). However, in a review of 432 array comparative genome hybridization (CGH) findings, over 37% of CNVs were inherited from a parent (Menten et al., 2006). We have found that studying the parents for duplications does not provide much helpful information in interpreting the results, as most are inherited. For example, of the unclear significance cases, interstitial duplications of 500 kb–1 Mb in size are de novo only 3% of the time (thus, 97% are inherited). Can it be assumed that the high likelihood of an inherited duplication means that the gain is benign?—perhaps so, but uncertainty may remain. Likewise, small deletions are more likely to be inherited than de novo, although many more of the deletions are de novo as compared to equivalent-sized duplications. Deletions of 500 kb–1 Mb in size were de novo in ~30% of cases, while deletions 250 kb–500 kb and less than 250 kb were de novo 20% and 10% of the time, respectively. Thus, the majority of small deletions are inherited. In another approach, we looked at gene content. The interstitial loss of 6–10 genes was de novo in 27% of cases, 3–5 gene deletions were de novo in 20% of cases, and deletions of 2 or fewer genes were de novo in 14% of cases. Thus, even with small deletions, the chance of finding a carrier parent is high; but the finding of transmission from a reportedly normal parent does not necessarily exclude the possibility that the deletion is pathogenic.

In addition, there are now examples of DNA CNCs that are associated with highly variable phenotypes, from abnormal with features in common (suggestive of a syndrome), abnormal with varying features, to a completely normal phenotype in carrier parents. These regions may represent susceptibility loci, but the conditions needed for a penetrant phenotype are unknown. To illustrate, we use deletion of 1q21.1 and deletion of 16p11.2 as examples. Duplications of these regions exist as well, but the phenotypes are less well characterized than those due to the deletions.

Deletions of 1q21.1, of 200 kb extent, are a necessary but not sufficient cause of the thrombocytopenia-absent radius (TAR) syndrome; it is proposed that some other genetic “modifier,” elsewhere in the genome, must coexist (Klopocki et al., 2007). In 75% of cases, the deletions are inherited from a clinically normal parent. In a screen of 5218 individuals with abnormal phenotypes (Mefford et al., 2008), 25 such deletions were identified; the phenotypes were quite variable, including mild-to-moderate mental retardation, microcephaly, cardiac abnormalities, and cataracts. Although not all parents were tested, six deletions were inherited from a clinically normal parent. This deletion was not found in a series of 4373 control individuals.

Deletions of 16p11.2 (not to be confused with the cytogenetically visible variant, p. 265), which in some publications have been described also as a CNV, have been associated with autism, as this abnormality appears to be enriched in that population (Weiss et al., 2008; Kumar et al., 2008). However, since this initial observation, several papers have shown that this microdeletion can be found in a wide variety of phenotypes (Bijlsma et al., 2009; Shinawi et al., 2010; Rosenfeld et al., 2010; Bochukova et al., 2010). Phenotypes include speech/language delay, cognitive impairment, motor delay, seizures, poor behavior, and obesity. A clear picture is yet to be drawn (and see also p. 321).

Genetic Counseling

The identification of chromosome abnormalities provides family members with an explanation for their child's developmental disability or birth defect, will allow for better information about recurrence risks, and will permit the anticipation of certain medical problems that may require intervention. The identification of genes causing the clinical features of chromosomal disorders may, in the future, lead to specific treatments. Microarray analysis increases the detection of clinically relevant DNA alterations in patients with developmental disabilities. The improved detection with this new technology needs to be balanced with the detection of results of unknown clinical relevance. Misinterpretation of CNCs as clinically causative, or the inability to provide explanation to parents of a finding of unclear clinical relevance, is not helpful and may be harmful if the family stops searching for the real answer to their child's problems.

Prenatal Diagnosis

Microarray analysis is gaining popularity in prenatal testing, and recent publications have demonstrated the benefits over traditional cytogenetic testing (Sahoo et al., 2006; Shaffer et al., 2008; Tyreman et al., 2009; Van den Veyver et al., 2009; Coppinger et al., 2009a; Faas et al., 2010). The use of microarrays in prenatal testing was recently addressed by the American College of Obstetricians and Gynecologists, which endorsed the use of targeted arrays as an adjunct to conventional cytogenetics in pregnancies with abnormal ultrasound findings (ACOG Opinion, 2009). For this purpose, microarrays should be specifically designed to identify known chromosomal syndromes, which minimizes the detection of CNCs of unclear clinical significance.

Prior to prenatal array-CGH, parents should receive comprehensive genetic counseling, including discussion of the inherent limitations of the test. The parents should be told the frequency of unclear results with the particular microarray that will be used, and they should be alerted to the possibility of needing parental samples for FISH or microarray analysis to aid in the interpretation of their prenatal test result. It needs to be understood by the physician, genetic counselor, and parents, that advances in technology may reveal findings about which little is known: their pregnancy might be identified to have a DNA alteration that has not been described before. In that case, little information can be provided about medical prognosis and the clinical spectrum of the disorder. For this reason, the selection of the array content and platform is a most important factor in the prenatal setting. In a study by Tyreman et al. (2009), 35 alterations were detected in 106 prenatal specimens; more than 37% of these were classified as CNCs of unclear clinical relevance, using a high-resolution SNP array. In contrast, microarrays designed for the purpose of prenatal testing should have far fewer unclear results (0.5%: Shaffer et al., 2008; Coppinger et al., 2009a). SNP-based arrays can reveal consanguinity or even incest, if unusually high levels of homozygosity are observed, and the family seeking array testing by this method should be aware of this (Schaaf et al., 2011). This is not a concern with array-CGH.

After the test results are available, the family will likely need additional genetic counseling depending on the findings. The genetic counselor will expect the laboratory to provide them with information about the alteration. Furthermore, there are public databases that can be of help in deciding the clinical relevance of certain CNCs. Examples are DECIPHER (http://decipher.sanger.ac.uk/application/), the Database of Genomic Variants (http://projects.tcag.ca/variation/), the database at Children's Hospital of Philadelphia (Shaikh et al., 2009), and the European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (Feenstra et al., 2006; ECARUCA, http://agserver01.azn.nl:8080/ecaruca/ecaruca.jsp) and the Signature Genomics Genoglyphix Chromosome Aberration Database (http://www.genoglyphix.com).

In the specific case of the 1q21.1 deletion associated with TAR syndrome (see earlier), demonstration of the cytogenetic defect can crucially inform counseling, our incomplete knowledge of the molecular pathogenesis not withstanding, as Uhrig et al. (2007) recount in being able to provide a clear diagnosis in a case of upper limb anomalies detected at fetal ultrasonography.

Notes:

[1] We can certainly expect advances in knowledge, and surely some CNVs will graduate to the status of pathogenic deletion or duplication. One example from many: a duplication close to the *PMP22* gene on chromosome 17p may influence its activity and lead to the syndrome of hereditary pressure-sensitive palsy (p. 323) (Weterman et al., 2010).

[2] Penetrance refers to the proportion of individuals with an imbalance that shows any trait resulting from that imbalance, whereas expressivity refers to the variability in phenotype of those who carry the imbalanced region.







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Chromosome Abnormalities and Genetic Counseling (4 ed.)

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Down Syndrome, Other Full Aneuploidies, and Polyploidy

Chapter: Down Syndrome, Other Full Aneuploidies, and Polyploidy
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IN THIS CHAPTER we consider the case of parents, themselves karyotypically normal, who have had a child, or a pregnancy that aborted, with a full aneuploidy or a polyploidy. Thus, we include the major trisomies (13, 18, 21) and sex chromosome aneuploidies (XXX, XXY, XYY, and 45,X) as well as less commonly seen autosomal aneuploidies and sex chromosome polysomies. The category of polyploidy is substantially devoted to triploidy. In the great majority, these defects arise from an abnormal event during meiosis or (in some triploidy) at conception. In a few, there is postzygotic generation of aneuploidy. Only in the case of parental gonadal mosaicism, or in the hypothetical setting of an apparent predisposition to meiotic error, will there apply an increased risk of recurrence of aneuploidy, over and above that associated with any parental age effect. Triploidy needs separate consideration.

Biology

Full aneuploidy is presumed in the great majority to be the result of meiotic nondisjunction. A diminished degree of meiotic recombination is typically observed, and this has led Hassold and Sherman (2000) to propose a two-hit sequence, the first hit being a less well-tethered bivalent at meiosis I, and the second hit being a consequential aberrant distribution at meiotic metaphase. Meiotic nondisjunction can happen at any parental age, but it is more frequent in older mothers. Alternatively, an abnormality has arisen in a premeiotic gametocyte, with the parent thus having a "wedge" of gonad that carries the abnormality (gonadal mosaicism). Such a parent would, of course, have an increased risk for only the one karyotypic defect. Finally, a small fraction of apparent full aneuploidy may be due to early mitotic nondisjunction in an initially 46,N conceptus with loss, or restriction to extra-embryonic tissue, of the normal cell line. For convenience, we note here also those forms of Down syndrome that are due to translocations (in most of which, however, the genetic imbalance is essentially the same as in the case of standard trisomy).

Autosomal Trisomy

Trisomy 21 (Down Syndrome)

Down syndrome (DS) is the archetypal chromosome disorder. It was the first medical condition shown to result from a chromosome abnormality, in 1959. It has for many years been recognized as the most common single known cause of intellectual disability, and it has the highest incidence at birth of any chromosome abnormality. Every counselor can expect frequently to deal with problems relating to DS and thus should be familiar with its genetics.

The Genotype to the Phenotype.

The DS phenotype—the characteristic facial appearance, body build, and mental defect—is due, in sum, to a triple amount of chromosome 21. Epstein (2002) provides a broad philosophical and historical as well as scientific review of the central role of the dosage effect in understanding the pathogenesis of the DS phenotype. Particular organ systems are particularly vulnerable, and Torfs and Christianson (1998) have identified characteristic malformations in a population study of nearly 3000 affected infants (Table 18–1). It is, in a sense, a "contiguous gene syndrome," in which there is an additional dose of an en bloc set of genes. The entire chromosome was sequenced by 2000, and the gene complement turned out to be surprisingly low, only 225 protein-coding loci in all (Hattori et al., 2000). This gene sparseness is plausibly a factor in the survivability of the trisomic state.

Table 18–1. Some Malformations Frequently Observed in Down Syndrome

MALFORMATION	RELATIVE RISK
Atrioventricular canal defect*	1009
Annular pancreas	430
Duodenal atresia	265
Patent ductus arteriosus*	152
Small intestinal atresia/stenosis	142
Ventricular septal defect*	95
Tricuspid valve defect*	84
Hypoplastic aorta*	77
Tetralogy of Fallot*	77
Atrial septal defect*	71
Ectopic anus	67
Cataract	54
Intestinal malrotation	45
Anal atresia/stenosis	34
Tracheo-esophageal fistula	26
Syndactyly	26

* Cardiovascular defect.

Source: Data from a population study in California 1983–1993, involving 2894 infants with Down syndrome (Torfs and Christianson, 1998).

It was logical that attempts be made to define those regions of the chromosome that might contribute predominantly to the DS phenotype: that is to say, to identify a "DS critical region" (DSCR), which might contain particular "DS genes." Such an attempt is illustrated in Figure **18–1**, with the region 21q22 having the predominant influence. The segment determining the facial features has been narrowed down by study of cases with partial duplications (Kondo et al., 2006), and two rather informative families are those described in Ronan et al. (2007) and in Eggermann et al. (2010b). In the former, a familial 3 Mb duplication in 21q22.13–q22.2 was associated with a clinically typical DS facies and a mild intellectual deficiency, while in the latter, a child with a 0.46 Mb duplication in a different part of 21q22 had no facial appearance to suggest DS (although he did present a Silver-Russell phenotype).

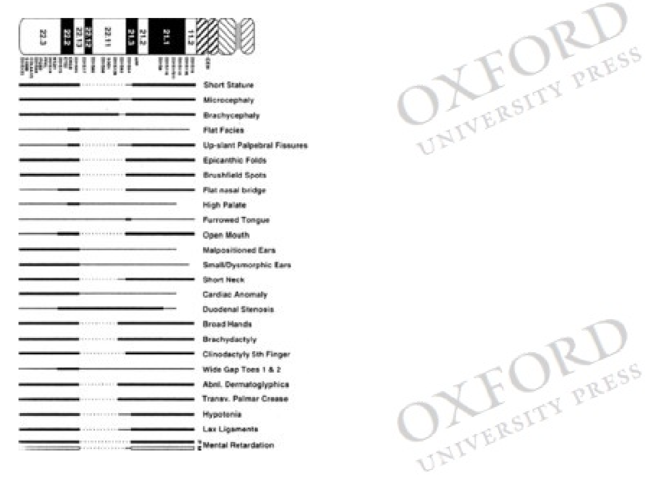




Figure 18–1
Phenotypic (trisomic) map of chromosome 21. Thick lines represent regions that must be trisomic to produce the particular trait. Thin-line regions may also contribute to that trait; the contribution of dotted-line regions is less clear. P, profound; M, mild. (From J. R. Korenberg et al., 1994, Down syndrome phenotypes: the consequences of chromosomal imbalance, *Proceedings of the National Academy of Sciences of the United States of America* 91:4997–5001. Courtesy J. R. Korenberg, and with the permission of the National Academy of Sciences.)

Pritchard and Kola (1999) propose that there are only a few loci whose 150% amount is central in the pathogenesis of DS, and that certain loci may make specific contributions to certain components of the phenotype. One dosage-sensitive gene for which a case has been made is *DSCR1*, whose very name indicates the presumption of a role. Overexpression of this gene in a mouse reduces the size of the hippocampus, and the number of dendritic spines in certain neurons within this part of the brain; and a

smaller hippocampal formation, and fewer dendritic spines, are seen in DS (Keating et al., 2008). And yet the duplication reported in Ronan et al., as mentioned earlier, did not include *DSCR1*, while that in Eggermann et al. did. *DSCR1* is one of a number of "*DSCR*" loci; each may have their individual contribution. The neural cell adhesion molecule *DSCAM*, which maps to 21q22.2-q22.3, is strongly expressed (in the mouse) in those equivalent regions of the brain that are compromised in DS, and this is an attractive candidate as a contributor to the DS brain phenotype (Barlow et al., 2001). Another mouse model is pointing to the genes *Olig1* and *Olig2*, mapping to 21q22.11, as candidates; these genes, in the 150% state, may lead to the overproduction of certain brain inhibitory interneurons (Chakrabarti et al., 2010). Duplication of the *APP* locus at 21q21 is presumed to be the cause of the early-onset dementia that is typically seen in DS, the consequence of a continuing *APP* overexpression, and hence an overproduction of β-amyloid, which is then deposited in Alzheimer-like plaques in the brain (Bird, 2008). This interpretation is well supported by the observations in the rare form of familial Alzheimer disease due to 21q21 duplication as an isolated genomic rearrangement (the duplications of size range 0.6–6 Mb) (Cabrejo et al., 2006). Other chromosome 21 genes may be of less critical effect when they are overfunctioning, and for some, perhaps many, it may make no difference at all.

Shapiro (1997) puts a somewhat different viewpoint, championing the "amplified developmental instability" hypothesis, and comments that "the search for a minimal region on chromosome 21 (the so-called DS critical region) responsible for producing DS has come full circle back to almost the entire chromosome." In his view, a direct role for one or a few single loci with a one-on-one gene-to-phenotype relationship is simplistic: "traits that characterize DS are complex, and should be viewed and analyzed accordingly." His general proposition is not unreasonable: that an excess of chromosome 21 encoded gene products perturbs the functioning of the products of many loci, from *all* chromosomes, in all manner of developmental and physiological pathways. Attempting to draw together the two viewpoints, the gene dosage theory and the amplified developmental instability theory, as do Neri and Opitz[1] (2009), we could suppose that the important genetic segments—the "DS loci"—may have their pathogenic role in the modulation, direct or epigenetic (Aït Yahya-Graison et al., 2007), of layer upon layer upon layer of cellular interactions that lead, as the end result, to a phenotypic *range* that is clinically recognizable as DS. "Complex" may be too simple a word to describe this.

What about the characteristic DS facies? Simply to observe one's fellows is enough to convince one that development of the human face must be the most subtle and complex and precise process. How could we begin to understand why the DS face is different, and recognizably so? Among others, one contributor may be the failure of the facial musculature to divide into its proper various components in fetal development, and this might have, of itself, a distorting effect upon soft tissue formation of the face (Bersu, 1980). Which gene in triple dose, or which combination of genes, might lead to such a process? Listing the genes that are on chromosome 21, and seeing which ones may or may not be associated with a component of the DS gestalt,[2] is merely another step on the way to knowing why this trisomy causes this phenotype.

Different Cytogenetic Forms

The usual basis of DS is standard trisomy 21 (Fig. **18–2**). The disorder has a number of other cytogenetic forms, and Figure **18–3** depicts the proportions graphically. Differences in the source and nature of the genetic errors underlying these various forms require each to be considered separately.

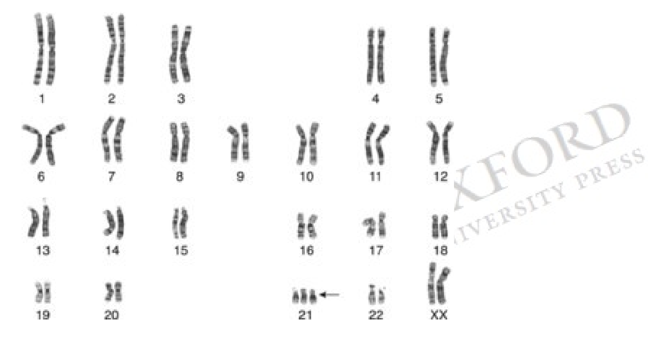


Figure 18–2
Karyotype of a child with standard trisomy 21.

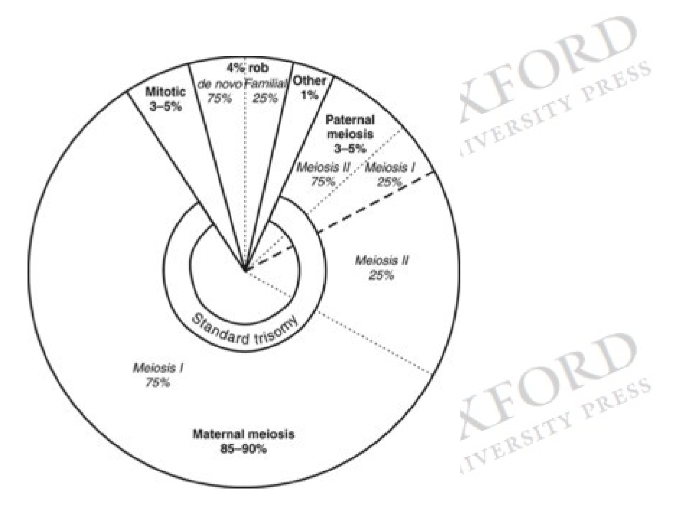


Figure 18–3
Origins of trisomy 21 (percentages rounded).

Standard Trisomy 21 Down Syndrome

The great majority (about 95%) of DS is due to simple trisomy of chromosome 21. Around 90% reflects a maternal meiotic error (Yoon et al., 1996). Three-quarters of these maternal errors occur at meiosis I, and one-quarter apparently at meiosis II, albeit that the latter may actually have been set up at meiosis I. Meiotic I errors are associated with reduced or actual absence of recombination between the chromatids of the chromosome 21 tetrad. Particularly an absence of recombination (with no chiasma forming, thus an "achiasmate" tetrad) may lead to each homolog being able to segregate without reference to the other, and thus without the imperative to move symmetrically. It seems probable that the biology of this meiotic susceptibility is universal across the human race (Ghosh et al., 2009).

Among the small fraction (about 10%) due to paternal errors, the proportions due to meiotic I and meiotic II errors are nearly equal. No usefully discernible paternal age effect

exists. As in the female, a reduced frequency of recombination observed in the meiotic I cases may underlie the cause of this male nondisjunction (Savage et al., 1998). Two asyet unexplained observations concerning trisomy 21 due to paternal meiotic errors are these: this fraction is a little greater among prenatally (11%) than postnatally (7%) diagnosed cases; and there is an excess of males among the DS offspring (Muller et al., 2000).

Standard trisomy DS typically occurs as a sporadic event, and recurrences are rare. These categories of cause of recurrence can be listed: gonadal mosaicism, a parental predisposition to nondisjunction, and chance.

Recurrence due to Mosaicism.

A trisomy 21 cell population in a parent (gonadal, or somatic-gonadal mosaicism) is presumed to be an uncommon cause of the production of disomic 21 gametes, although perhaps less rare than originally thought (Bruyère et al., 2000; Mahmood et al., 2000; Kuo, 2002). Pangalos et al. (1992b) studied 22 families in which trisomy 21 had occurred more than once (in siblings, in second- and in third-degree relatives), applying DNA polymorphism analysis. Parental gonadal mosaicism was proposed as the cause of sibling recurrence in 5 of 13 families (about 40%); but other than these, chance alone was enough to explain the recurrences in most, if not all families. James et al. (1998) studied four women, each of whom had had three trisomy 21 conceptions. Two of the mothers were under age 35 at the time of the trisomic conceptions, and they both showed a very low-level mosaicism (0.5% and 4% on blood karyotyping). Neither had a DS phenotype. One of these mosaic mothers was shown herself to have originated as a trisomic conceptus, but an early mitotic loss of a chromosome 21 almost entirely corrected the karyotype. The other two women were older, and in their case it was more likely that the multiple trisomic conceptions occurred independently, as maternal age-related events. In their collaborative series from six Japanese clinics, Uehara et al. (1999c) record the exceptional case of a couple having had five successive pregnancies with trisomy 21 (one DS child, four diagnoses at amniocentesis). Both parents had normal karyotypes on blood and skin analysis. It would seem rather probable that one parent may have had fully trisomic gonadal tissue. Sachs et al. (1990) followed 1211 pregnancies at prenatal diagnosis, subsequent to the occurrence of trisomy 21 in a previous pregnancy, and observed six recurrences (for a rate of 0.5%). In two of these instances, mosaicism was shown. One father karyotyped as 47,+21/46,N on skin analysis; and one mother showed trisomic cells in 3%, 14%, 44%, and 47% on culture of, respectively, blood and skin, and—in a more direct observation—of each ovary.

Ovarian biopsy proved the point in a mother of three DS children (and one normal child) who typed 46,XX on peripheral blood, but in whom 8 out of 20 ovarian cells showed trisomy 21 (Tseng et al., 1994). Other similar examples are on record. Nielsen et al. (1988) report a couple having had six documented pregnancies with standard trisomy 21, and five other unkaryotyped pregnancies ending in neonatal death or abortion. The mother typed 46,XX on peripheral blood, and 46,XX/47,XX,+21 in ovarian somatic cells. (Even if the oöcytes were all or nearly all 47,+21, it remains perplexing that no known 46,N conception occurred.) An in vitro fertilization (IVF) setting enabled analysis of the gametes themselves in a woman studied by Cozzi et al. (1999). She had a normal and a DS child at ages 29 and 32, and then had prenatal diagnoses of trisomy 21 at 32 and 36 years. No trisomic mosaicism was detected on peripheral lymphocyte analysis. At IVF, of seven embryos, four were trisomy 21 and one tetrasomy 21, with only two showing normal disomy 21. Four unfertilized oöcytes were analyzed, and three had a supernumerary chromosome 21.[3] A rather elegant demonstration of maternal gonadal mosaicism is described in Cupisti et al. (2003), who, in the study of a woman presenting for fertility treatment, identified three oöcyte-polar body pairs having one copy of chromosome 21 in the egg, and two copies in the first polar body. As for the male, Hixon et al. (1998) analyzed sperm samples from 10 men who had fathered a DS child, the additional chromosome 21 having been demonstrated to be of paternal origin. None showed any increase in the fraction of sperm with disomy 21.

Recurrence due to Nondisjunctional Tendency.

Do some (nonmosaic) individuals, for a certain biological or environmental reason, run an increased risk of producing a trisomic 21 conception? Could a specific sequence within chromosome 21 influence its disjunction (Gair et al., 2005)? Are some people susceptible to a dietary deficiency? Is there a range of “meiotic robustness” in the population? These are perfectly respectable concepts, albeit that they remain quite hypothetical. If so, what possibilities might there be? Several theories for a general predisposition to aneuploidy have been put forward, and some of these are discussed on p. 57. While some of these various possibilities may be more plausible than others, they are all speculative, and we conclude that there is at present no routinely practicable basis enabling the counselor to identify, ahead of time, those parents whose risk is high, and those whose risk is low, to have a second pregnancy with trisomy 21.

Recurrence Risk Estimates after One Affected Child or Pregnancy.

The earliest estimates of risk are due to Penrose (1956),[4] prior to the discovery of the chromosomal basis of DS, and to Stene (1970). Penrose proposed the risk of recurrence to be “doubled, or perhaps nearly trebled” compared to the general population risk, irrespective of maternal age; while Stene derived a figure of 1% for mothers under age 30, with no increase in the age-specific risk for those over 30, at the time of birth of their DS child. More sophisticated analyses were subsequently enabled by the collection of amniocentesis data, and from population studies; and Warburton et al. (2004), Morris et al. (2005b), and De Souza et al. (2009) have determined estimates on the basis of extensive data sets. The advice set out later in the “Genetic Counseling” section is based upon this work. It does remain true that for younger mothers the recurrence risk is, in absolute terms, small.

Recurrence Risk Estimates after Two Affected Children/Pregnancies.

When a couple have had two (or more) trisomic 21 conceptions, one has to assume an increased risk applies to a subsequent pregnancy, quite possibly a “substantial” risk. The recurrence may well have been due to gonadal mosaicism.

Occurrence Risk Estimates with Down Syndrome in a Second- or Third-Degree Relative.

More widely in the family, it appears that a history of standard trisomy DS in second- or third-degree relatives does not, in the main, imply an increased risk (Hook, 1992; Pangalos et al., 1992b). Berr et al. (1990) assessed 188 families in which a DS child had been born, and there were comparable numbers of DS cases among the second- and third-degree relatives, and in the relatives of 185 control families.

Mosaic Down Syndrome

47,+21/46,N mosaicism accounts for about 2% of individuals with clinically diagnosed DS. Mosaicism results from a malsegregation of homologs, or an anaphase lag of one homolog, occurring postzygotically. Some individuals with mosaic DS arise from initially trisomic 21 zygotes, losing one of the chromosomes 21 by anaphase lag (Fig. 3–8c in Chapter 3). Others may arise from normal conceptuses, with nondisjunction producing 45,–21/46,N/47,+21 mosaicism, with the 45,–21 line thereafter lost (Fig. 3–8a). Pangalos et al. (1994) studied 17 families in which there was a child with mosaic trisomy 21, and 10 children had three chromosome 21 alleles, indicating their origin from a trisomic conceptus. The chromosome 21, which was subsequently lost to enable formation of the 46,N cell line, showed no predilection for being a maternal or paternal homolog. The remaining seven mosaics had no evidence of a “third allele,” and distinction in these between an initially 46,N or 47,+21 conception was not possible. Whatever the basis, for practical purposes counseling needs to proceed as though the child has standard trisomy 21, recognizing that this will overestimate the risk in some. Genetic counseling for the mosaic individuals themselves is covered on p. 234.

Isochromosome 21 Down Syndrome

After standard trisomy 21, this is the most common chromosomal category of DS. It has often been called a “21q21q Robertsonian translocation,” but in fact the two 21q components are usually identical, from the same parent, and thus isochromosome is the more accurate term, and the karyotype is more accurately 46,i(21q) (Robinson et al., 1994; Ruiz-Casares et al., 2001). In one series of 112 de novo “rob(21q21q)” probands, none of 130 full sibs and 34 half-sibs had DS (Steinberg et al., 1984). Nevertheless, three of the parents actually showed a low-grade mosaicism, and presumably their having had an affected child reflected that the 21q21q cell line was included in the gonad. Indeed, a few examples of recurrence in subsequently born siblings are recorded, and parental gonadal mosaicism is the presumed basis of such recurrence (Sachs et al.,

1990; Kovaleva and Shaffer, 2003; Chen et al., 2009). This is a point that Mark et al. (1977) directly proved in one case: a woman having sequential pregnancies with the karyotype 46,i(21q) herself typed 46,XX/46,XX,i(21q) on ovarian fibroblast analysis (but 46,XX on blood). Hall (1985) offers the cautionary story of a mother given a low risk of recurrence, who went on to have a second affected child from a second marriage (on resampling of her, a single 46,XX,rob(21q21q) cell was found in 100 cells analyzed). Given this desirability of distinguishing between postzygotic and meiotic (gonadal mosaic) mechanisms, with their differing counseling implications, Kovaleva and Shaffer (2003) advocate study with polymorphic markers in this (and other) homologous Robertsonian translocations.

Robertsonian Translocation Down Syndrome

Almost all translocation DS concerns a Robertsonian translocation (discussed in detail in Chapter 7). About one-quarter of Robertsonian translocation DS is familial and three-quarters is de novo (1% and 3% of all DS, respectively).

De Novo Robertsonian Translocation Down Syndrome.

Both parents, by definition, have normal chromosomes. The abnormal chromosome may usually arise as a sporadic event in maternal meiosis I, from a chromatid translocation (Petersen et al., 1991). Such mutational events are rare and, in the great majority of families, recurrences are not seen. But gonadal mosaicism remains a possibility. The so-called rob(21q21q) is, in most cases at least, actually an isochromosome (see earlier).

Familial Robertsonian Translocation Down Syndrome.

One or the other parent (almost always the mother) is a translocation heterozygote and has transmitted the translocation, in an unbalanced state, to the DS offspring.

Down Syndrome with Reciprocal Translocation

The DS phenotype is substantially due, as we noted earlier, to a duplication of the chromosome segment 21q22.2-q22.3. A reciprocal translocation involving chromosome 21 has the potential to produce, in a gamete from the heterozygote, a duplication of the DS critical region, whether from 2:2 or 3:1 meiotic segregation. The unbalanced adjacent-1 karyotype from the t(18q;21q) illustrated in Figure 5–15 (second row) in Chapter 5 is an example. Or interchange trisomy 21 may result (Fig. 5–13 in Chapter 5). These translocation scenarios are extraordinarily rare, the cause of less than 0.1% of DS. Scott et al. (1995) describe a child with DS from a maternal t(12;21)(p13.1;q22.2), and Nadal et al. (1997) and Lee et al. (2005) describe similar cases from a paternal translocation and insertion, respectively. (It is from studies of cases of partial trisomy 21, comparing those with typical DS and those with different phenotypes, that phenotypic maps, as in Figure **18–1**, can be drawn; Kondo et al., 2006.) Interchange trisomy 21 was reviewed by Dominguez et al. (2001), with a total of only 23 published families being accumulated.

Other Chromosomal Forms of Down Syndrome

A number of chromosomally distinct forms of DS result from specific structural changes to chromosome 21.

(1) The least rare of these is the terminal rearrangement that produces a mirror-image chromosome around the telomeric region (Pfeiffer and Loidl, 1982). The chromosome has two centromeres, one of which is usually inactive, and satellites on both ends. Such chromosomes are always the result of sporadic mutational events, possibly the result of a translocation between sister chromatids (Pangalos et al., 1992a).
(2) DS is seen occasionally in association with other aneuploidies, almost always a sex chromosome aneuploidy, such as 48,XYY,+21 and 46,X,+21; this is known as double aneuploidy. It is usually the result of a double event of nondisjunction resulting in one abnormal gamete. Rather less likely is a scenario of separate events in gametogenesis in both parents.

"Interchromosomal effect" has been invoked in standard trisomy DS in the setting of a parental karyotypic abnormality not involving chromosome 21 (e.g., a 13;14 Robertsonian translocation or a reciprocal translocation). In other words, might it be that the rob or the rcp in some way perturbed the distribution of the chromosome 21s? The answer in fact seems to be no, with the case for interchromosomal effect remaining tenuous at best, although a possible exception might be the Robertsonian translocation in the setting of oligospermia (see pp. [link], [link]).

Trisomies 13 and 18 (Patau Syndrome and Edwards Syndrome)

These syndromes are much less frequent than DS (about 1 in 12,000 and 1 in 6000 live births for trisomies 13 and 18, respectively), and both show a maternal age effect. As with trisomy 21, correlative phenotypic mapping allows certain segments of chromosomes 13 and 18 to be implicated in the genesis of certain phenotypic traits observed in these syndromes (Tharapel et al., 1986; Epstein, 1993; Boghosian-Sell et al., 1994). On molecular studies in trisomy 18, over 90% reflect a maternal meiotic nondisjunction. Uniquely, nondisjunction is considered to happen most frequently at the second meiotic division, this division not taking place until the short period of time surrounding the process of fertilization (Bugge et al., 1998), although there was a contrary view from Verlinsky's group. From the direct analysis of polar bodies, chromosome 18 meiosis I errors outnumbered those in meiosis II (Verlinsky et al., 2001a). In about 90% of trisomy 13, the additional chromosome is of maternal origin, with meiosis I and II equally susceptible (Bugge et al., 2007); in at least some mosaic cases, the causes may be similar (Jinawath et al., 2011).

Recurrence of trisomy 18 had been recorded in one or two single case reports, and one or two instances of recurrence, or none at all, had been seen in earlier prenatal diagnostic series or retrospective surveys (Pauli et al., 1978; Ferguson-Smith, 1983; Stene et al., 1984; Baty et al., 1994; Uehara et al., 1999c). Baty et al., (1994) noted a 39-year-old mother having had prenatal diagnosis of trisomy 18 at age 39, and a liveborn trisomic 13 infant at age 40 years. No case of trisomy 13 recurrence had been recorded. It had seemed, on balance, that no discernible increased recurrence risk existed, with chance and maternal age the main factors. These may still be the main factors: but in Warburton et al.'s (2004) more recent review, a true increased risk, albeit a very small one, has emerged from the analysis of a very large body of prenatal diagnosis data, and this assessment was confirmed by De Souza et al. (2009). This is dealt with in more detail in the section on "Genetic Counseling."

Other Autosomal Trisomy

It is extremely rare for any other autosomal trisomy to survive through to (or near to) term. About two dozen examples of each of trisomy 9 and 22 are known, and nonmosaic trisomies 7, 8, 10, and 14 are represented by only one or two reports (Schinzel, 2001; Brizot et al., 2001; Tinkle et al., 2003; Póvoa et al., 2008).

In contrast, trisomies are very common in miscarrying pregnancies, a matter dwelt upon in detail in Chapter 23. Robinson et al. (2001) considered that recurrences of trisomy (even of the same trisomy) in spontaneous abortion might represent no more than a common thing happening again, and this may often be the case. Nonetheless, Warburton et al.'s large review (2004) comprised sufficient data to demonstrate that a previously karyotyped trisomic spontaneous abortion indeed does marginally increase the risk for a potentially viable trisomy at a subsequent prenatal diagnosis (see "Genetic Counseling" section), thus indicating, in some instances at least, that an individual predisposition may have been the cause.

Autosomal Monosomy

Many autosomal monosomies are presumed to end in arrested growth in the first few mitoses, prior even to the time of implantation, with some possibly proceeding to the stage of "occult abortion" (p. 382), their existence unknown had it not been for the window of observation afforded by preimplantation diagnosis. The single exception may be monosomy 21, albeit that most earlier reports of monosomy 21 have since been reinterpreted as being due, for the most part, to an unbalanced translocation involving chromosome 21 (Cardoso et al., 2008). One presumed case was identified at 17 weeks of pregnancy, going on to fetal death in utero early in the third trimester, although again

the cytogenetic diagnosis was not beyond doubt (Chang et al., 2001; Phelan, 2002).

Sex Chromosome Aneuploidy

XXY (Klinefelter Syndrome), XXX, XYY

These aneuploidies occur at roughly similar frequencies, about 1 per 1000 of the appropriate sex. About 75% of XXX and about 40% of XXY Klinefelter syndrome (KS) is due to a maternal meiotic error, and in three-quarters of each of these it is the first meiotic (MI) division that is involved, this MI group showing a maternal age effect. It is noteworthy that almost half of KS results from a paternal MI error (MacDonald et al., 1994). Fathers of paternally originating KS may have marginally elevated levels of disomic XY sperm in comparison with fathers of maternally originating cases, possibly reflecting an inherent tendency among a small minority of these men to produce aneuploid sperm (Eskenazi et al., 2002). In what may have been the only known example of a recurrence, Woods et al. (1997) report two XXY brothers. The karyotype in both reflected a paternal meiosis I error. Manifestly, XYY of meiotic origin must be due to a paternal error, at MII. All three sex chromosomes aneuploidies can have a postzygotic mitotic generation, which may present as mosaicism.

45,X Turner Syndrome

In about three-quarters of TS it is the paternal X chromosome that is absent (Hassold et al., 1990a; Uematsu et al., 2002). Mostly, the error is a meiotic one and resides in paternal gametogenesis, possibly reflecting an absence of pairing along most of the X-Y bivalent with a consequential vulnerability in the process of disjunction (Jacobs et al., 1997). Fathers of nonmosaic $45,X^{m}$ Turner girls may have a marginally increased risk to produce sperm nullisomic for a sex chromosome. Martínez-Pasarell et al. (1999) analyzed sperm from four fathers and eight controls, and there was a slight increase in 24,XY sperm (0.22%) and nullisomic sperm (0.48%) in the fathers compared to the fractions in controls (0.11% and 0.32%, respectively). This might suggest that some fathers of nonmosaic $45,X^{m}$ Turner girls have a slight proneness to produce sperm nullisomic for a sex chromosome; but if so, the near-absence of recurrences would point to a very minor influence.

An alternative explanation is that the loss occurred postzygotically, and the “45,X” child is actually a 45,X/46,XX mosaic, with a very low proportion of XX cells, but this is apparently an uncommon event (Jacobs et al., 1997). Wiktor and Van Dyke (2005) describe 22 patients with apparently nonmosaic 45,X in whom, upon further study, three had a minor XX cell line, and 19 were apparently pure 45,X; no XY cell lines were seen. To the contrary, Uematsu et al. (2002) suggest that most TS may actually be due to a structurally abnormal gonosome (X or Y) having been generated in paternal meiosis, with a 46,X,abn(X) conception resulting, and subsequent mitotic loss of the abn(X) leaves a 45,X karyotype.

These theories notwithstanding, the observational data point to a very low recurrence risk. In the literature review of Kher et al. (1994), they could find only one instance of 45,X recurrence in sisters. From the Birth Defects Register of Victoria, Australia, over the period 1995–2008, of 245 prenatal diagnoses of 45,X, in none had the indication been of a previous chromosome abnormality (J. L. Halliday, Murdoch Childrens Research Institute, personal communication, 2010). In the case of a postzygotic origin, if it could be presumed to have been an event that occurred at random in a single mitosis in the early embryo, the risk of recurrence would be regarded not to be raised at all. Kher et al. did however report a unique family with occurrence of 45,X/46,XX in sisters.[5]

Rare Polysomies

Polysomies such as XXXX, XXYY, XYYY, XXXY, XXXXX, and XXXXY are very rare. Successive nondisjunctions in one parent, the other contributing a single sex chromosome, is the mechanism in most if not all (Hassold et al., 1990; Deng et al., 1991). Apart from the extraordinary circumstance of (hypothetically) a familial tendency to mosaicism, these polysomies arise sporadically (Bergemann, 1962; Kher et al., 1994). Rare reports of coincidence with some other aneuploidy in the family may more likely reflect chance than a causal link (Court Brown et al., 1969).

Polyploidy

Triploidy

The chromosome count in triploidy is 3n = 69, with a double (2n) chromosomal contribution to the conceptus from one parent (Fig. **18–4**). Triploidy can reflect diandry or digyny, with the double contribution coming from the father or mother, respectively (also referred to as Types I and II triploidy) (Fig. **18–5**). The great majority of triploid conceptions abort during the first or early second trimester. Of those aborting at the embryonic stage, most are digynic, while in contrast, most fetal losses reflect a diandric state (McFadden and Robinson, 2006). The appearances on morphological examination at the stage of the embryo do not differ according to a digynic versus diandric origin, whereas the clinical presentations are readily distinguishable by the fetal stage of development, the latter distinction possibly reflecting a placental-mediated influence.

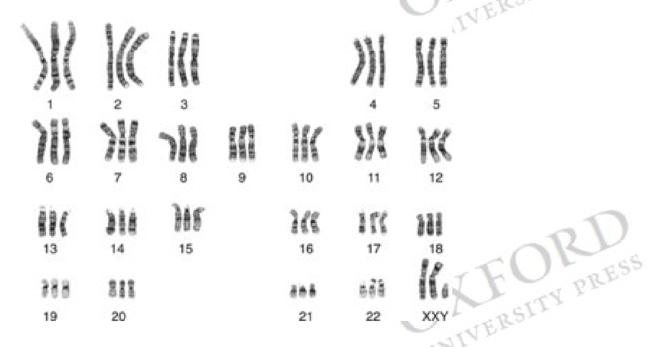


Figure 18–4
Karyotype of a 69,XXY triploid fetus (see also Fig. 23–4 in Chapter 23).

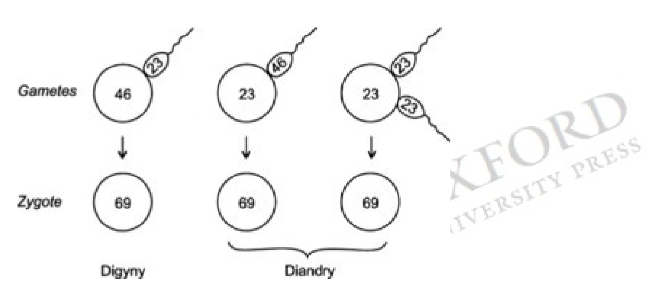




Figure 18–5
The three major routes whereby triploidy may arise. A complete failure of a meiotic division produces a diploid egg (*left*) or sperm (*middle*). Simultaneous fertilization by two sperm is dispermy (*right*).

The Two Distinct Forms of Triploidy

Diandry is usually, almost always, the consequence of dispermy; that is, two sperm simultaneously fertilizing the ovum (Zaragoza et al., 2000; McFadden et al., 2002).[6] The fundamental problem in this instance may lie in the "zona reaction," which is the response of the investing shell of the ovum, the zona pellucida, to prevent further sperm entering after the first has penetrated. A minority are due to fertilization with a diploid sperm, this diploid state having arisen from a complete nondisjunction in spermatogenesis.

Digyny is most commonly due to a diploid egg, which may be the result of nondisjunction of the entire chromosome set at either the first or the second meiotic division in oögenesis, meiosis II being the more vulnerable, or, of the fertilization of a primary oöcyte[7] (Zaragoza et al., 2000; McFadden and Robinson, 2006). A rare cause may be the fusion of two eggs (whimsically called "dieggy"). Individual susceptibility may exist, as discussed later. Diploidy can be presumed to exist in the "giant binucleate oöcyte," and these visibly abnormal gametes have actually been shown at IVF to lead to a triploid embryo (Balakier et al., 2002; Rosenbusch et al., 2002).

Natural History.

Triploidy is not uncommon in early pregnancy (1%–3% of recognized conceptions), but about 99.99% are lost as first-trimester miscarriage or second-trimester fetal death in utero. From Hawaiian data, of 38 recognized triploid pregnancies over the period 1986–1999, around 40% were XXX, 60% XXY, and a single case of XYY. Most (80%) aborted early, a few (10%) presented as fetal deaths in utero, and 10% were electively terminated (Forrester and Merz, 2003a). Of all 16-week pregnancies, only 1 in 30,000 are estimated to be triploid, and at 20 weeks, only 1 in 250,000 (Snijders et al., 1995).

Diandric triploids mostly abort in the 10- to 20-week period, the mean at 12 weeks. The very few diandric triploid pregnancies that survive to the second trimester typically show partial hydatidiform mole; growth retardation is usual but not invariable (Daniel et al., 2001). *Dygynic* triploids mostly abort early (mean 10 weeks), although those exceptional few that remain are able to continue through to the third trimester, when they come to outnumber diandric cases. These surviving digynic triploids develop as a severely growth retarded fetus with marked head-body disproportion, the head being relatively large, and with an abnormally small and nonmolar placenta (McFadden and Langlois, 2000; Daniel et al., 2001). In one case of a digynic 69,XXX triploid coexisting with a normal 46,XY twin, survival to 20 weeks (when selective feticide was done) may have been supported by the normal fetus (Gassner et al., 2003). Intrauterine survival may also be promoted if there is fetal-placental karyotypic discordance, with the placenta being diploid (Kennerknecht et al., 1993a). Survival to the third trimester is associated almost invariably with perinatal death. Of those liveborn, hardly any digynic triploids survive for more than a month; there is one extraordinary instance of death not until 312 days (Sherard et al., 1986; Hasegawa et al., 1999).

Recurrence

While most triploidy occurs sporadically, a genetic predisposition does exist, and recurrences are well described. In one case, the cause was laid at recurring maternal meiosis II errors: a woman coming to IVF, having had two previous triploid pregnancies, had triploidy identified by preimplantation genetic diagnosis (PGD) in 2 out of 13 conceptions (Pergament et al., 2000). In this example, a recurrent maternal meiosis II error[8] could be implicated, as might also be so in a similar case in Check et al. (2009): since fertilization had been achieved with a single sperm at intracytoplasmic sperm injection (ICSI), diandry was clearly excluded. More difficult to explain is the occurrence of triploidy of both digynic and diandric bases to the one couple, and the occurrence of both partial hydatidiform mole (due to diandric triploidy) and complete mole (the rare type associated with biparental disomy) to the same couple (Kircheisen et al., 1991; Deveault et al., 2009). A role for the *NLRP7* gene is emerging. These aspects are discussed in more detail in Chapter 23.

Diploid/Triploid Mosaicism.

Van de Laar et al. (2002) accumulated 25 cases from the literature and reported three of their own. These three came from a population catchment of 15 million over a 20-year period, attesting to the rarity of the condition. The triploid line typically reflects digyny, and the basic mechanism may be inclusion of the second polar body at a very early stage after conception of a diploid zygote. Similarly in diandric cases, the mechanism may be dispermy, but with one sperm pronucleus sequestered in the cytoplasm for a few divisions before being incorporated into the nucleus (Daniel et al., 2003b; Wegner et al., 2009). Daniel et al. refer to "delayed digyny" and "delayed dispermy," respectively, as the course of events whereby the extra pronucleus sits to one side, so to speak, while the diploid lineage is in the process of being established, and the pronucleus then being taken up into the nucleus of one blastomere to give rise to the triploid cell line. Survival of the affected fetus in utero is presumably promoted by the diploid cell line. In most cases the triploid line is not seen on a blood analysis, and fibroblast culture is necessary (Boonen et al., 2011). A single instance of a false-negative amniocentesis is to be noted (Flori et al., 2003).

Rare Complexities.

"Hypotriploidy" describes the circumstance of a 68-chromosome constitution. The usual mode of formation may be fertilization of a diploid egg with a 22,–X sperm, leading to a 68,XX karyotype; the phenotype resembles that of digynic triploidy (Pasquini et al., 2010).

45,X/69,XXY mosaicism is recorded in a single case, an infant presenting with genital ambiguity, and who displayed complete soft tissue syndactyly of the index and middle fingers of one hand (this being a feature of triploidy) (Quigley et al., 2005). On blood, the karyotype was nonmosaic 45,X, and on skin fibroblast culture, 45,X[3]/69,XXY[77]. The authors propose an initial 46,XY zygote, which lost an X in one cell at possibly the first cell division, giving rise to the 45,X lineage, followed by delayed dispermy of a (or the) 46,XY cell, to give the 69,XXY cell line.

Tetraploidy

A number of mechanisms may lead to a conceptus with 92 chromosomes, four of each homolog (4n = 92). The simplest, and most usual, may be reduplication of the diploid set in the zygote: at the first mitosis, the chromosomes replicate, but the cell fails to divide. These would karyotype as either 92,XXXX or 92,XXYY. Such a mechanism could not explain the very rare case of 92,XXXY, and here it is necessary to invoke such scenarios as retention of a polar body with concomitant dispermy, trispermy, or dispermy with a haploid and a diploid sperm (Baumer et al., 2003; Surti et al., 1986). In one instance, a woman having PGD for recurrence of digynic triploidy had one 92,XXXX embryo, possibly suggesting a meiotic susceptibility in her case (Check et al., 2009). The typical phenotype is that of miscarriage with complete hydatidiform mole, or "hydropic abortion" (Fukunaga, 2004)

Tetraploidy in a term pregnancy is exceedingly rare, and survival in one apparently nonmosaic case to 26 months unprecedented (Teyssier et al., 1997; Guc-Scekic et al., 2002). Mosaic diploidy/tetraploidy in a person has been described in association with severe mental defect, and it may only be detectable on skin fibroblast study (Edwards et al., 1994). A complex case is that reported in Leonard and Tomkins (2002) of a retarded woman with body asymmetry and hypomelanosis of Ito, in whom some fibroblasts cultured from hypopigmented skin showed 92,XXXX, others being 46,XX and 46,XX, t(1;6)(p32; q13), and 46,XX on blood.

True diploid/tetraploid mosaicism may be quite frequent at the blastocyst stage of development, but either the abnormal embryo is cast off shortly thereafter or, especially if the proportion of tetraploid cells is small and the blastocyst is otherwise of good quality, the polyploid component may be confined to the trophoblast and in due course come to comprise a minor fraction of placenta (Clouston et al., 2002; Bielanska et al., 2002b). Possibly for this reason, tetraploidy can occasionally be seen at chorionic villus sampling

(CVS) and at amniocentesis, reflecting a “normal” tetraploidy of part of the placenta, with the remaining extrafetal and fetal tissues being karyotypically normal (Benkhalifa et al., 1993). Alternatively, tetraploidy at prenatal diagnosis may be artifactual.

Genetic Counseling

Down Syndrome

The central requirement for accurate genetic advice in DS is knowledge of the chromosomal form in the affected family member. If a child diagnosed as having DS has died and no chromosome studies were performed, it may be reasonable to check for the possibility of a familial translocation in the consultand(s).

Previous Child with Standard Trisomy 21 (Including Mosaicism)

If the child has standard trisomy 21, or is a 47,+21/46 mosaic, it is unnecessary routinely to study the parents’ chromosomes. One can assume, with considerable confidence, that they will type as 46,XX and 46,XY. The risks for recurrence of trisomy 21 in a subsequent amniocentesis, or occurrence of a different aneuploidy, are summarized as follows, and as set out in detail in Tables 18–2 and 18–3, and with reference to the studies of Warburton et al. (2004), Morris et al. (2005), and De Souza et al. (2009).

Table 18–2. Increases in Recurrence Risk, Given as Multiples Compared with the Maternal Age-Related Baseline, for Women Who Have Had a Previous Trisomic Pregnancy

PREVIOUS ABNORMAL PREGNANCY	FOLD INCREASED RISK OF RECURRENCE FOR:	
	SAME TRISOMY	OTHER VIABLE TRISOMY
Trisomy 21 at maternal age <30 current maternal age <30	8.2×	2.4×
Trisomy 21 at maternal age <30, current maternal age ≥30	2.2×	2.4×
Trisomy 21 at maternal age <35	3.5×	1.3×
Trisomy 21 at maternal age ≥30	1.6×	1.7×
Trisomy 21 at maternal age ≥35	1.7×	1.5×
Trisomy 13 overall	8.6–9.5×	1.5×
Trisomy 18 overall	1.7–3.1×	1×
Trisomy 13 or 18 at maternal age <35	7.8×	1.6×
Trisomy 13 or 18 at maternal age ≥35	2.2×	1×
Trisomies 13, 18, XXX and XXY	2.3×	1.6×
Nonviable trisomy in spontaneous abortion*		1.8×

Notes: Separate figures are given for the risk of recurrence of the same trisomy (“homotrisomy”) or of a different trisomy (“heterotrisomy”). If wished, the appropriate multiple for a particular case can be applied to the woman's current age-related risk, as listed in Tables 24–2, 24–3, 24–4, 24–5 in Chapter 24, in order to generate an adjusted recurrence risk figure. Figures are from the prenatal data of Warburton et al. (2004) and pre- and postnatal data of De Souza et al. (2009), and they are grouped in various ways, according to the formats of these papers. Specific age-related figures for previous trisomy 21 are also given in Table 18–3, column B.

* But cf. Robinson et al. (2001), who discerned no increased risk following an aneuploid miscarriage.

Table 18–3. Estimates of Recurrence Risk for Trisomy 21, According to the Mother's Current Age (Column A), and According to Her Age at the Birth of the Affected Child (Column B)

A. BASIC AGE-SPECIFIC RISK		B. ADDITIONAL RISK DUE TO PREVIOUS DS	
MATERNAL AGE AT THIS CURRENT PREGNANCY	RISK (‰)	AGE AT THE EARLIER DS PREGNANCY	RISK (‰)
20	0.9	20	6.2
21	0.9	21	6.2
22	0.9	22	6.1
23	0.9	23	6.0
24	0.9	24	5.8
25	1.0	25	5.7
26	1.0	26	5.4

27	1.1	27	5.2
28	1.1	28	4.8
29	1.2	29	4.4
30	1.4	30	4.0
31	1.6	31	3.5
32	1.9	32	2.9
33	2.3	33	2.4
34	2.9	34	1.9
35	3.7	35	1.5
36	4.9	36	1.1
37	6.6	37	0.8
38	8.8	38	0.6
39	11.7	39	0.5
40	15.2	40	0.4
41	19.2	41	0.3
42	23.5	42	0.2
43	27.8	43	0.2
44	32.0	44	0.2
45	35.8	45	0.2
46	39.2	46	0.1
47	42.1	47	0.1
48	44.5	48	0.1
49	46.4	49	0.1
50	48.0	50	0.1

Notes: Risks A and B are then to be summed. This combined risk figure relates to the probability of detection of trisomy 21 at early second-trimester amniocentesis. For example, a woman who is now pregnant, and due to deliver at age 30 (risk = 1.4‰ from column A), and who had had a DS pregnancy when she was 25 (additional risk = 5.7‰ from column B), has an overall risk for trisomy 21 in the current pregnancy of 1.4 + 5.7 = 7.1‰, or 1 in 141. Note how, with advancing maternal age at the current pregnancy (A), the additional risk component due to having had a previously affected child (B) progressively diminishes; in other words, at these older ages, the maternal-age factor becomes the overwhelming contributor to the risk.

DS, Down syndrome; ‰, per 1000

Source: From Morris et al. (2005b).

- For a mother under 30 years old at the birth of the DS child, and who is still under age 30, the risk for recurrence of trisomy 21 at amniocentesis is about eight-fold (8.2×) her age-related risk.
- If a mother who was under 30 at the birth of the DS child is now over age 30, her current age is the predominant risk factor, and the additional risk due to the previous affected pregnancy is about two-fold (2.2×) her age-related risk.
- If a mother was over 30 at the birth of her DS child, the current age-related figure becomes the more important factor, especially as she gets into her later thirties and forties, and the extra risk due to the previous DS child increases her age-related risk only by about one and a half-fold (1.6×) (and see Table 18–3, column B.).
- The risk of recurrence at birth will be a little less than at prenatal diagnosis, reflecting a greater likelihood for natural loss of a trisomic pregnancy in the period following the time of amniocentesis.
- The risk for a different viable trisomy (amniocentesis diagnosis) is a little above double the age-related risk (2.4×) for younger women under 30 at the previous DS pregnancy, and a little below double the age-related risk (1.7×) for those 30 or over at the DS pregnancy. Since the baseline figures are very low (see Table 24–4 in Chapter 24, p. 408), the absolute risk figure is still a low one.
- If 35 years, rather than 30, is used as the cutoff age for younger/older mothers, a slightly different perspective is given (Table 18–2).

In any event, regardless of the exact figure, the practical point is that the risk for a recurrence of DS is comfortingly low, only approaching the 1% mark by the mid-thirties. Nevertheless, most couples seek the reassurance of prenatal diagnosis in pregnancies after having had a child with DS. Elkins et al. (1986b) observe that some of these parents declare they would not abort a trisomy 21 fetus, and the counselor needs to be sensitive to possible ambivalent feelings of the parents in this setting.

Two Previous Trisomic 21 Conceptions

One can only offer an educated guess that the risk for a third trisomic conception will be “substantial.” A skin biopsy study would be largely academic. If gonadal mosaicism (rather than de novo recurrence) is the cause, a considerable fraction of whichever gonad it is must be involved, since two separate samplings have already come from this fraction. A risk in the range of 10%–20% may be a fair figure to offer. Preimplantation genetic diagnosis would have an obvious attraction.

Isochromosome 21 Down Syndrome

From the 0/164 fraction among siblings of de novo isochromosome 21q DS in Steinberg et al.'s series (1984), the risk for recurrence is presumed to be small. Nevertheless, three parents (3%) in this series were demonstrably mosaic, and there are a handful of recurrences otherwise on record; a cautious stance is thus prudent. A risk figure in the region of 1%–2% may be a reasonable one to offer.

Previous Child with Robertsonian Translocation Down Syndrome

Obviously, distinction between de novo and familial forms of translocation DS is crucial; this distinction is made by chromosomal studies of the parents. For the de novo translocation, a recurrence risk figure of <1% is applicable (Gardner and Veale, 1974). In the case of *familial* Robertsonian translocation DS, the genetic risk for the female carrier is substantial. The risk to have a liveborn child with translocation DS is about 10%, while the likelihood to detect translocation trisomy 21 at amniocentesis is about 15%. For the male carrier, the risk to have a child with translocation DS is small, about 1% (and see Chapter 7).

Previous Child with NonRobertsonian Translocation Down Syndrome

In the rare instance that translocation DS is associated with a familial reciprocal translocation, the principles presented in Chapter 5 are to be followed.

Previous Child with Other Chromosomal Category of Down Syndrome

For sporadic structural changes such as the terminal rearrangements, the risks are presumed to be very low (less than 0.5%). For the double aneuploidies, there is no evidence to suggest the risks are any different from the recurrence risks for standard trisomic DS.

Wider Family History of Down Syndrome

There is no conclusive evidence of an increased risk for second- and third-degree relatives of individuals with standard trisomic DS themselves to have offspring with the condition. The appropriate action in the setting of “a family history of DS” is to determine whether the affected member has standard trisomy 21. If this is so, the family may be reassured that there is no discernibly increased risk, which advice could also reasonably be offered if a single case was associated with older maternal age. If the karyotype of the index case is unknown, and the mother had been younger, the small possibility of a familial translocation may be checked by chromosome study of the counselee.

Trisomy 21 in Products of Conception

The finding of trisomy 21 in products of conception after spontaneous abortion (in those centers where this testing may be done) presents a problem. Should this, for genetic counseling risk assessment, be regarded as equivalent to having had a child with DS? From about 10 weeks gestation through to term, about a third of trisomic 21 conceptions are lost (p. 405), and it may be stochastic events in utero rather than intrinsic genetic differences that distinguish those that abort and those that survive. It may be prudent to err on the side of caution and provide a risk figure as though the abortion had been a liveborn child.

Trisomy 13 and Trisomy 18, and Other Autosomal Trisomy

Recurrence of trisomy 13 or 18 is a very rare observation. Nevertheless, in Warburton et al.'s (2004) data, a true increase in risk did emerge, albeit that the absolute figure will still be very small, given the very small baseline age-related figures. In the study of De Souza et al. (2009), based upon 748 women who had had a previous trisomic 13 or 18 pregnancy, a similar increase in risk was observed. The numbers were small (albeit the largest such study ever undertaken), and of 1057 subsequent pregnancies, recurrent trisomy 13 or 18 was diagnosed in 9. Relative risk estimates from these two studies are displayed in Table 18–2.

If there is an increased risk for a different potentially viable trisomy (“heterotrisomy”), it must be very small, and Warburton et al. (2004) derive a 1.6-fold factor. In De Souza et al. of the 1057 pregnancies just noted, 8 had trisomy 21, but this number was scarcely different from expectation based upon age. In the case of a previous pregnancy with some other type of autosomal trisomy (typically identified in products of conception following spontaneous abortion), from Warburton there may be an increased risk (1.8-fold), but very small in absolute terms, for a potentially viable trisomy in a subsequent pregnancy, although from Robinson et al. (2001), no such risk was discerned. It seems likely that many, if not most cases of recurrent trisomy in older women, whether in spontaneous abortions or in live births, represent the increased risk of a trisomic conception related to maternal age as the key predisposing factor.

XXX, XXY, XYY, 45,X, Other Sex Chromosome Aneuploidy

There is no firm evidence that a recurrence risk above the age-specific figure exists, and indeed in respect of XXX and XXY no recurrences, of either homotrisomy or heterotrisomy, were observed in the study of Warburton et al. (2004). Prenatal diagnosis is discretionary.

Triploidy

Diandric triploidy associated with partial hydatidiform mole has a ~1% risk of recurrence; we discuss this in more detail on p. 400. As noted in the “Biology” section, some women may have a predisposition for digynic triploidy. However, the level of risk for recurrence of triploidy, or occurrence of an aneuploidy, must usually be small, since in the series of Robinson et al. (2001) no increased risk was discernible, for women having had more than one previous spontaneous abortion due to triploidy (or aneuploidy), to have yet another chromosomally abnormal pregnancy. Prenatal karyotyping and/or early pregnancy ultrasonography may reasonably be offered.

The prevention of chromosomal pathology, as a direct exercise, largely involves secondary prevention: in essence, the selective termination of pregnancies in which a chromosomal abnormality has been identified, or the discarding of abnormal embryos following PGD. Primary prevention is indirect, and encouraging a younger maternal age may be the only feasible approach, absent any clear understanding of environmental factors that might compromise the chromosomal integrity of gamete or zygote. But one remarkable exception to this state of affairs concerns the actual correction of a chromosomally abnormal zygote: and this involves the diandric triploid zygote, otherwise destined to undergo implantation failure or, in the minority that actually implant, to proceed to a severe fetal defect. A triploid zygote due to dispermy will possess three pronuclei. In vitro removal of one pronucleus, at IVF, would restore normality. This would have to be, in the case of dispermy, one of the paternal pronuclei, thus leaving one maternal and one paternal pronucleus. Escribá et al. (2006) applied this approach to tripronuclear embryos in the research laboratory, removing the pronucleus farthest from the second polar body (the one closest to the polar body being very likely maternal), and followed the embryo through to the blastocyst stage. They were able to confirm restoration of diploidy, and could also observe that these corrected embryos showed normal development at day 5, unlike the uncorrected embryos, in which no inner cell mass was seen to form.

And in the first ever example of “chromosomal cure” of a child-to-be, Kattera and Chen (2003) corrected a tripronuclear zygote, implanted the embryo, and a normal 46,XY boy was subsequently born. These authors comment, cautiously, that this approach should be used “only as a last resort.” In contrast, Pergament (2010) boldly predicts that, by 2020, we will fully understand the mechanisms of meiosis, and we will be able to “treat oöcytes, sperms and preimplantation embryos to ensure that the euploid state will be obtained at conception and then maintained during early embryonic development,” initially doing this in vitro, but eventually in vivo. We shall see.

Tetraploidy

True (that is, not artifactual) tetraploidy is too rare for a clear picture to have emerged. Sporadic occurrence would seem very probable.

Notes:

[1] Their review celebrates the fiftieth anniversary of the discovery of the chromosomal cause of DS, and provides a fascinating discussion of nineteenth- and twentieth-century thinking leading up to this event.

[2] Gestalt: “a configuration or pattern of elements so unified as a whole that it cannot be described merely as the sum of its parts.”

[3] Two of the no. 21 chromosomes had identical haplotypes, indicating that the mother's mosaicism was due to postzygotic error in an initially normal 46,XX conception (Fig. 3–8a).

[4] His paper was entitled “Some Notes on Heredity Counselling,” and he also referred to “genetical counselling,” one of the first uses of this expression.

[5] A possible role due to the fragile X full mutation in predisposing to X/XX mosaicism is noted on p. 253.

[6] Dispermy could be deduced simply from the cytogenetic analysis in the case reported in Lim et al. (2003), the man carrying a translocation 46,XY,t(2;6)(p12; q24). The 69,XXY mole had both the balanced translocation and one unbalanced form, reflecting fertilization with one sperm from alternate segregation and the other from adjacent-1.

[7] One extraordinary case was due to the fertilization, by a normal haploid sperm, of a first polar body, which carried a diploid chromosomal set (Bieber et al., 1981). Although acardiac, in utero survival of the triploid fetus was enabled by its normal co-twin, who had arisen from a normal fertilization of the egg. The egg and its first polar body (PB1) had respectively haploid and diploid constitutions as depicted in Figure 3–1 (in Chapter 3), lower right.

[8] At the same PGD procedure, 3 of the 13 embryos were trisomic 18, and as noted earlier, this trisomy is mostly due to a meiosis II error.

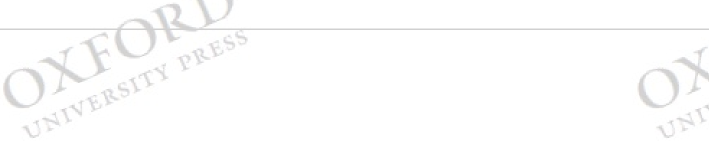






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Chromosome Abnormalities and Genetic Counseling (4 ed.)

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Structural rearrangements

Chapter: Structural rearrangements
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IN THIS CHAPTER we consider the circumstance of parents, themselves typically karyotypically normal, who have had a child in whom a structural chromosome rearrangement has been identified. Under this heading, we distinguish in particular deletions (partial monosomy) and duplications (partial trisomy). If the rearrangement occurs during meiosis, or at a postzygotic mitosis, we generally assume a recurrence risk no different from the general population. These cases arise anew—de novo—with the affected child. If, however, the rearrangement arises at a premeiotic mitosis, the parent would be a gonadal mosaic, and an increased risk for recurrence, for a second child with the same abnormality, could in theory apply. Usually, no prior distinction between these two possibilities can be made, although how often a rearrangement is observed (unique/nonrecurrent, or commonly seen/recurrent) may suggest the site of generation (see later). Here we consider those deletions and duplications in which cytogenetic or molecular cytogenetic techniques are important in demonstrating the defect, and which are generally thought of as being chromosomal conditions. In some, and more particularly those now coming to light through the microarray, there is a possibility that a parent might be a carrier, and so our focus is not exclusively on de novo defects.

Some of these deletions and duplications occur sufficiently frequently, and/or present a sufficiently distinctive phenotype, that they have acquired syndrome status. The classical route whereby a chromosomal syndrome came to be established followed the recognition of a group of patients with a very similar clinical picture, often with a characteristic dysmorphology: "phenotype-first." Subsequent cytogenetic studies revealed the underlying chromosomal basis in common (in the case of Down syndrome, this took nearly a century). Nowadays, the typical approach is "genotype-first," or "reverse dysmorphology" (Shaffer et al., 2007; van Ravenswaaij-Arts and Kleefstra, 2009). Subtle deletions and duplications may not present a distinctive enough phenotype that would allow the clinician to "call" a syndrome. But in the laboratory, recurrent rearrangements, whether seen in house, or in collaboration with other cytogenetic services, nationally or internationally, can be collected. It is then up to the clinicians to draw together the observations from the patients thus identified and to construct the core features of the new syndrome. This new approach of identifying the chromosomal abnormality first can reveal the natural clinical variation of the genomic rearrangements, which might never have been possible with the traditional phenotype-first approach.

Biology

Mechanisms of Formation of Structural Rearrangement

Human chromosomes are disconcertingly dynamic structures. Genomic DNA can be deleted, duplicated, and moved around, with untoward results. Conditions that are the consequence of these rearrangements in underlying genomic structure or architecture may be referred to as "genomic disorders" (Lupski, 2009). Older expressions, not to be discarded, include "partial aneuploidies," "segmental aneusomies," and "contiguous gene disorders." This is in clear contrast to the classic "pure" whole chromosome aneuploidies, in which no rearrangement has taken place.

These genomic rearrangements are typically generated from the operation of a chromosomal repair process. Different categories of rearrangement can be considered, according to whether the repair process is homology-dependent or homology-independent; whether the event takes place during meiosis or in a mitotic cell; and whether the recombination is within a chromosome (intrachromosomal) or between chromosomes (interchromosomal).

Nonallelic Homologous Recombination

The common basis for many of these rearrangements, and more so the recurring ones, lies in the existence of multiple DNA sequences throughout the genome, generally of some thousands of base pairs, which are sufficiently similar ("paralogous," rather than exactly homologous) that they enable the erroneous coming-together of different chromosome regions. Within the two sequences (inter- or intrachromosomal) involved in a particular exchange, there is a length of perfect or near-perfect homology, and this is the site of the actual strand exchange (nonallelic homologous recombination [NAHR]). Studies on the Charcot-Marie-Tooth region of 17p12, and the Smith-Magenis syndrome region in 17p11.2 (Fig. **19–1**), in particular, have informed this work (Lupski, 2009). These sequences, whose misalignment during meiosis sets the stage for the recombination between the two regions, have been dubbed "duplicons," segmental duplications, or low copy repeats (Gu et al., 2008). Some paralogous sequences run in opposite directions; and in that case, paracentric microinversions, existing as normal polymorphisms, make it possible for this recombination event to take place. The syndromes of duplication or deletion of 15q13.3 (see later) in particular have been well studied in this respect (Sharp et al., 2008).

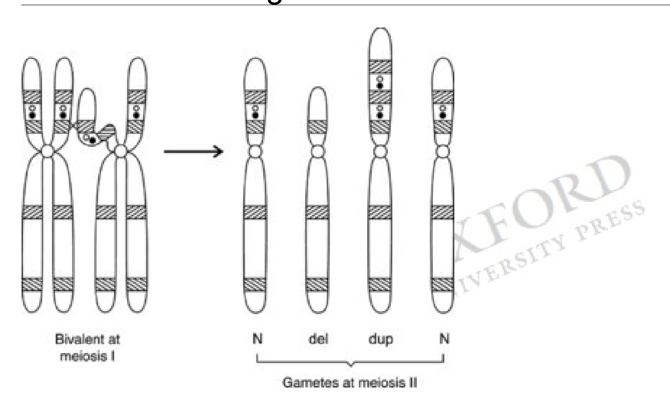




Figure 19–1
One mechanism to produce a duplication and a deletion (and see text) is based upon the promiscuous activity of similar DNA sequences, “duplicons,” that exist at numerous places along the chromosome (shown as crosshatched segments). Consider a segment between two such duplicons, indicated here by the black and white spots (imagine these to be two fluorescence in situ hybridization [FISH] probes). Misalignment of the two duplicons, followed by nonallelic homologous recombination within them (×), produces recombinant products that are reciprocally imbalanced: one with a deficiency of the chromatin between the two duplicons, and the other with a duplication. The deletion chromosome is shown with no black or white spot, while the duplication chromosome has a double set of black and white spots in tandem. The general case is drawn after Chandley (1989). The genotype in the child (normal, deletion, duplication) resulting from such meiotic recombination will depend upon which of the chromatids ends up as the homolog in the gamete. The two classic examples both reside in chromosome 17. Smith-Magenis syndrome and Potocki-Lupski syndrome are due to deletion and duplication, respectively, for the segment 17p11.2p11.2. On a smaller scale, and just a little further up 17 short arm, a 1.7 Mb segment within 17p12 including the *PMP22* gene is deleted in hereditary pressure-sensitive neuropathy and duplicated in Charcot-Marie-Tooth neuropathy.

Nonhomologous End Joining

During mitotic cell division (and including in the premeiotic gametocyte), aberrant DNA replication and repair may lead to the generation of a rearrangement. Different chromosomal segments may happen to be in close proximity, due to their “geographical space” within the nucleus; or upstream or downstream sequences with similarity (such as *Alu* repeats) may have predisposed to their coming together (this more so in the case of deletions). Then, if breaks occur during replication, instead of the correct ends being brought back together, the broken ends of different segments may inappropriately be ligated. This is nonhomologous end joining (NHEJ). If two segments share a few nucleotide sequences in common (“microhomologous patches”), this may of itself predispose to apposition and recombination: “microhomology-mediated end joining.” Or the active replication fork may “stall” and switch to a template of similar microhomology elsewhere on the same or another chromosome, which happens to be in the same “space” within the nucleus. This is “fork-stalling and template-switching”; and as well as simple deletions and duplications, this may be the basis of some more complex rearrangements, such as triplications, and deletion/duplication combinations (Zhang et al., 2009a). In contrast to NAHR, these mechanisms apply particularly to deletions and duplications seen only in nonrecurring cases (Vissers et al., 2009).

Origin Pre-, Intra-, or Postmeiosis

As just noted, the recurring de novo deletion or duplication is considered typically to originate at *meiosis*, and the child is nonmosaic. Nonrecurring abnormalities, in contrast, may have arisen at a *premeiotic mitosis*, such that the parent is a gonadal mosaic, with a “wedge” of the gonad containing the abnormal chromosome constitution (note that the child would be nonmosaic). In fact, a second affected child of such a parent is scarcely ever seen, and one might conclude that any such wedge would comprise only a very small fraction of the gonad. Nevertheless, it is usually appropriate to check the parental karyotypes (as well as for the reason of reassurance), in order to test the possibilities that one parent may be either a carrier of a balanced rearrangement, or a low-grade mosaic for the abnormal chromosome (the normal cell line in a phenotypically normal parent being, presumably, predominant in the soma). An example is illustrated in Figure 3–12 (in Chapter 3) of an interstitial deletion del(1)(q25q31.2) which was identified at amniocentesis, and which led to the discovery of 46,XY[20%]/ 46,XY,del(1)[80%] mosaicism on blood karyotyping of the father, thus revealing him to be a somatic-gonadal mosaic. Normal parental karyotypes do not absolutely exclude the possibility of such mosaicism, as exemplified in two sisters with a chromosome 16 deletion whose parents' karyotypes on blood were normal (Hoo et al., 1985), and in a case of recurrent, interstitial 1p36 deletion in two sisters from a gonadal mosaic mother, who did not have any evidence on blood fluorescence in situ hybridization (FISH) analysis of the deletion (Gajecka et al., 2010). A fuller discussion of parental gonadal mosaicism is given on p. 54.

A rearrangement arising at a *postzygotic mitosis* would lead to mosaicism in the child, generally for a normal and for the abnormal cell line. Two major scenarios warrant consideration, in the case of an abnormal child in whom such mosaicism is diagnosed. First, the conceptus is chromosomally normal, and at a subsequent mitosis an abnormality is generated that gives rise to a karyotypically abnormal cell line, along with the 46,N line. The karyotype becomes 46,N/46,(abn). This category will typically have no increased risk for recurrence. Second, from an initially 47,+(abn) conceptus, a postzygotic “correction,” with loss of the abnormal rearranged chromosome, generates a normal cell line, and so the karyotype becomes 46,N/47,+(abn). Here, the recurrence risk will essentially be that of the 47,+abn state. With the exception of marker chromosomes, somatic mosaicism for a structural rearrangement is rarely recognized (Leegte et al., 1998; Zaslav et al., 1999).

Simple and Complex Deletions

The simple scenario of a “clean-cut” deletion may in some, indeed many instances, be an oversimplification. Davies et al. (2003) restudied a group of 16 deletion patients, and in 3 the supposed deletion proved to be a rearrangement, involving subtelomeric regions. Gunn et al. (2003) studied a child initially karyotyped as 46,XY but whose clinical features suggested an 18q deletion. This was indeed proved, but the deletion seemed rather small given the severity of the phenotype. Using FISH and microarray analysis, they could show that a segment from distal 4q had been inserted into the site of the 18q deletion, giving a partial 4q trisomy along with the partial 18q monosomy. And many examples now exist whereby terminal deletions, apparently simple on light microscopy, prove to be complex rearrangements upon molecular investigation, with submicroscopic duplications, triplications, and inversions, in addition to the deletions (Ballif et al., 2003b, 2007b). Zuffardi et al. (2009) propose that many “deletions,” according to cytogenetic analysis, are actually inverted duplications contiguous with terminal deletions. These result from dicentric chromosomes (which can form either through NAHR or NHEJ), which then undergo a breakage-fusion-bridge cycle (Ballif et al., 2003b), with formation of a telomere at the site of rupture. This view is supported by Rowe et al. (2009), who suggest that the most usual mechanism may operate at a premeiotic meiosis, due to NHEJ.

Complementary Deletion/Duplication

The rare complementary deletion/duplication offers insight into the likely site of generation of this particular rearrangement. If the del/dup should arise at the very first somatic replication following conception, two countertype cell lines will be produced at the two-cell stage, with no normal cell line. In the event that extrafetal tissues can be studied, and still no normal cell line seen, the interpretation of a first-mitosis scenario is strengthened; thus, Rodriguez-Revenga et al. (2005) could draw such a conclusion from their prenatal diagnostic case of dup(18q)/del(18q) mosaicism, the chorionic villi showing both karyotypes, although on amniocentesis and fetal blood, only the del(18q) was present. If one of the cell lines is of lesser viability, a child might show the complementary karyotypes at birth, but later in childhood, only one cell line. Morales et al. (2007a) report an example, an abnormal infant who as a newborn had dup(7)(q21.1q31.3)[90]/del(7)(q21.1q31.3)[10] mosaicism, but upon restudy at age 12 and 14 months, only the

dup(7) cell line was seen, looking at blood and exfoliated urinary tract epithelial cells. If the del/dup arises at the second (or subsequent) mitosis, there will be a normal cell line as well; and Tharapel et al. (1999) illustrate this circumstance in a child initially identified at amniocentesis, undertaken upon the basis of a choroid plexus cyst and echogenic bowel. In this child, the normal cell line was present in about half of cells, with the remaining cells containing either a deletion for 7p11.2-p13 or a duplication for this segment.

Influence of Sex of Parent

Certain duplication/deletion rearrangements may have a predilection for happening in the parent of one or other sex. Chromosome 17p11.2 rearrangements are more often of paternal origin, and they may be intrachromosomal or interchromosomal in their generation. The nearby 17p12 region is also more susceptible to rearrangement in the paternal gonad, although in contrast to 17p11.2, paternal duplications/deletions are always interchromosomal, and the uncommon maternal cases are all intrachromosomal (Potocki et al., 2000a). The X chromosome has a particular vulnerability in the male, perhaps because it is largely unpaired at meiosis, and it can refold up and down its length (Giglio et al., 2000). The 1p36 deletion varies in size, and the larger deletions are more often of paternal generation (Gajecka et al., 2007). We may also note that de novo Robertsonian translocations are almost always maternal in origin (Bandyopadhyay et al., 2002), while on the other hand, the great majority of de novo apparently balanced translocations and complex rearrangements arise in the fathers (Grossmann et al., 2010; Thomas et al., 2010). The frequency of paternal deletions and duplications shows a small increased age effect, and with some chromosomes being more susceptible than others (Templado et al., 2011).

The Need for a Telomere

Respecting the requirement that integrity of the telomere be maintained, some mechanisms of terminal deletion need to include a process to restore the telomere (Ballif et al., 2000a; Daniel et al., 2008). If the terminal deletion is interstitial (thus, actually subterminal), then the original telomere simply remains intact. If, however, the telomere is lost in the deletion process, a neo-telomere can be generated ("telomere healing"). If another chromosome is involved in the process, its telomere can be "captured" to fulfill the requirement. Ballif et al. (2003) made particular study of the 1p36 deletion (see later discussion) and showed that all three mechanisms were involved, the commonest being the acquisition of a telomere from another chromosome.

Deletion

We have traveled a distance from the earliest days of cytogenetics when the first deletion was published, which was large enough to be seen on a solid-stained "B group chromosome" and associated with cri du chat syndrome (Lejeune et al., 1963). We have, now, a spectrum from large deletions ("classical cytogenetic deletion syndromes"), through microdeletions detectable only since the use of high-resolution banding, to deletions beyond the range of banding but detected on combined molecular/cytogenetic (FISH) or purely molecular methodology (array comparative genomic hybridization [array-CGH]), and deletions which are so small that only a single gene is removed. Examples of the general karyotypic form of an interstitial deletion, in any chromosome A, are 46,del(A)(p21p23) or 46,del(A)(q12q12).

Contiguous Gene Syndrome

Recollect that loci are arranged in linear order along a chromosome. Often there is no apparent reason for the order: the nonsignificance of the contiguity of two loci has been likened to the unimportance one would attach to "Appalachian Mountains" being next to "apple" in an encyclopedia. Our genome differs from an encyclopedia in that about a third of all the entries relate to one topic: the development and functioning of the brain. Many of the other entries (loci) relate to the control of morphogenesis during embryonic life. If a length of chromosome is deleted, a sequence of adjacent (contiguous) genes will be lost. The phenotype resulting from this can be described as a contiguous gene deletion syndrome (Schmickel, 1986; Tommerup, 1993). In almost any deletion detectable cytogenetically, some of the deleted loci will be brain loci, while others could be for anything, but probably including some morphogenesis loci. Thus, we have the classic clinical picture in deletion syndromes of intellectual deficit of some degree, dysmorphism, and organ malformation. The deletion produces a monosomy—or "haplo-insufficiency"[1]—for the region of the chromosome that has been removed, and loci in this segment are underexpressed. Proof that genetic expression is reduced by 50%, for example, in the case of the 18q–syndrome (see later), was adduced by Wang et al. (1999b) in measuring mRNA from a number of 18q loci.

Some of the loci whose haplo-insufficiency contributes to the phenotype in the various deletion syndromes are beginning to be defined, as noted in individual entries in the "Genetic Counseling" section. It seems likely that many such genes will have their untoward outcome not in a simple one-to-one relationship with a single gene product, but rather in a complex layering and interlacing of consequential effects. As yet, however, it is only the simple case that we can begin to understand: such as, for example, the brain white matter abnormality of the 18q– syndrome just mentioned, that is presumably a direct consequence of the loss of a structural myelin gene on 18q. As array-based CGH may define deletions/duplications of quite small extent, only a few genes may be located within the particular segment, and the counselor blessed with a scientific curiosity has the opportunity to check which genes these are, and perhaps to make an informed speculation (which some parents might find helpful) as to which of these might have contributed to a child's phenotype (and see section on "Gene Discovery" later).

If two or more mendelian disorders coexist in the one person, a contiguous gene deletion is a strong possibility. In order to prove the point, molecular methodology can be brought to bear; or a direct chromosomal test using FISH offers an immediate visual demonstration of the deletion. We have, for example, seen a young woman presenting with a history of recurrent bacterial infections since childhood, and night blindness and diminishing peripheral vision since teenage, leading to diagnoses of chronic granulomatous disease and retinitis pigmentosa (Coman et al., 2010b). The X-linked forms of these conditions being very closely linked, a contiguous gene deletion suggested itself, and a FISH probe targeted to a DNA sequence between the two loci was generated. Its nonhybridization to one X chromosome essentially confirmed the supposition of a deletion. Furthermore, this led the way to another diagnosis, that of a partial protein intolerance, due to deletion of the *OTC* (ornithine transcarbamylase) gene, which lies in the same Xp region.

> An infrequent mechanism for abnormality is that the deletion may "unmask heterozygosity" for an autosomal recessive disorder, and there are a handful of examples of this having been recognized (Coman and Gardner, 2007). Flipsen-ten Berg et al. (2007) reported an infant with Wolf-Hirschhorn syndrome (WHS) due to a deletion on one chromosome 4p, and who then went on to develop, over and above the WHS, signs of Wolfram syndrome (diabetes mellitus, diabetes insipidus, deafness, optic atrophy). The *WFS1* locus for Wolfram syndrome is on chromosome 4 short arm, which led these workers to examine the gene on the "normal" chromosome 4. A point mutation in *WFS1* was discovered. The 4p deletion on the other chromosome allowed this mutation to be "exposed"; and the child, being essentially hemizygous, got the syndrome. Similarly, a child with a 22q13 deletion (Phelan-McDermid syndrome), and having an *ARSA* mutation on the other chromosome 22, would develop the fatal recessive brain disease, metachromatic leukodystrophy (Bisgaard et al., 2009).

Subtelomeric Deletions.

The subtelomeric region is typically gene-rich and may be more prone to deletion. A very subtle deletion may submit only to FISH and molecular methodology, and somewhere in the vicinity of 2%–10% of patients with "unclassified multiple congenital anomalies/mental retardation" (MCA/MR) may prove to have a subtelomeric deletion. Microarray is the more powerful of the two methodologies, in terms of the yield of abnormalities (Ballif et al., 2007c). A less affected parent might have the same deletion, and thus parental studies may be warranted (notwithstanding a note referring to "parents normal" on the test requisition form). Some apparent subtelomeric "deletions" may actually be due to normal polymorphism (Chapter 16), and this is another reason for parental chromosomes to be studied, along with careful clinical assessments, carefully interpreted (Ballif et al., 2000a).

Microdeletions and Mendelian Disorders.

Several examples exist of mendelian syndromes, the majority of cases representing classic single-gene mutations, but a few being the consequence of haplo-insufficiency due to microdeletion that deletes the entire gene, and often neighboring genes as well. Rubenstein-Taybi syndrome (see later) was one of the earliest to exemplify this scenario

(the gene *CBP*); and Charcot-Marie-Tooth disease type 1A and hereditary pressure-sensitive palsy provide an instance of both duplications and deletions with respect to a single gene (*PMP22*) (see later), and indeed this genomic mechanism being more frequent than classical mutation at this locus. Other cases, most of which are also noted later, include: Pitt-Hopkins syndrome (the gene *TCF4*), Sotos syndrome (*NSD1*), Alagille syndrome (*JAG1*), Saethre-Chotzen syndrome (*TWIST*), Greig syndrome (*GLI3*), one type of holoprosencephaly (*SHH*), CHARGE syndrome (*CDH7*), one type of lissencephaly (*LIS1*), and X-linked ichthyosis (*STS*). If neighboring genes are also deleted, this may contribute to a wider phenotype; or these other genes might not be dosage sensitive, and the clinical picture is essentially the same as in the mendelian case. A number of these loci were originally discovered due to patients having deletions of the regions concerned.

Gene Discovery.

Taking a broader view, deletions can point the way to discovery of novel genes coding for particular organs and tissues. A deletion that, from clinical observations, has a particular clinical association with clefting, may remove, say, contiguous genes w, x, y, and z. It could reasonably be imagined that haplo-insufficiency of one of these genes (perhaps gene x, if its product had a theoretical role in cell-cell interaction) could contribute to the cause of cleft lip; and then it could further be assumed that the normal role of gene x is in contributing to the process of lip formation during early embryogenesis. Brewer et al. (1998) have reviewed some hundreds of small deletions listed in the Oxford Cytogenetic Database, correlating the malformations with which these deletions have been associated. Patterns have emerged: some deletions seem particularly likely to lead to a heart defect, while others may be prone to cause clefting. A larger scale map, addressing only the distal long arm of chromosome 13, is shown in Fig. **19–2**. This cartographic approach, broad-brush in the 1990s, we may expect to become progressively more finely drawn as this new century advances.

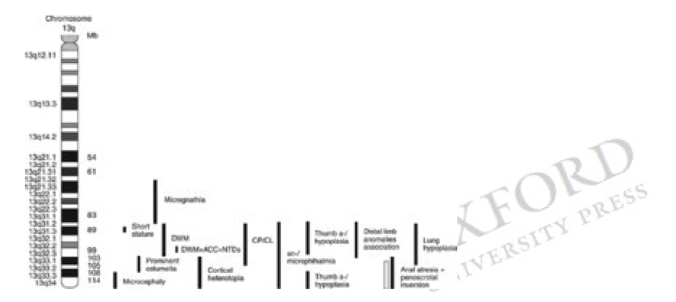


Figure 19–2

A deletion-phenotype map for deletions of distal 13 long arm, from 13q21.1-qter. The application of microarray comparative genome hybridization (CGH) allowed considerable precision in the delineation of the deleted segments. ACC, agenesis of corpus callosum; CP/CL, cleft palate with or without cleft lip; DWM, Dandy-Walker malformation; NTDs, neural tube defects. (From M. Kirchhoff et al. 2009, Phenotype and 244k array-CGH characterization of chromosome 13q deletions: An update of the phenotypic map of 13q21.1-qter, *American Journal of Medical Genetics* 149A: 894–905. Courtesy M. Stefanova, and reproduced with the permission of Wiley-Liss.)

Researchers hoping to find genes directing development of the heart, or genes controlling lip formation, could focus their searches in these chromosomal regions. Similarly, nephrogenesis genes and neurogenesis genes may come to light in the analysis of deletions associated with renal maldevelopment and epilepsy, respectively (Singh et al., 2002a; Amor et al., 2003). Different syndromes with a phenotypic trait in common can clarify the nature of the genetic heterogeneity. Thus, multiple exostoses are a component of the Langer-Giedion syndrome, due to deletion at 8q24, and the Potocki-Shaffer syndrome, due to 11p11.2 deletion, consequent upon haplo-insufficiency of different bone growth genes at these two segments.

Rare Complexity.

A rare (or rarely recognized) complexity is a change in size, from parent to child, of a subtelomeric deletion. Faravelli et al. (2007) observed a mother having a 1.5 Mb 4p deletion, which expanded in size to produce a 2.8 Mb deletion in her child with typical Wolf-Hirschhorn syndrome; in retrospect, the mother had subtle signs to suggest a *forme fruste*. Similarly, South et al. (2008a) describe a normal mother with a de novo 18q subtelomeric deletion of 0.4 Mb, which increased in size nearly 10-fold, to 3.7 Mb, in her abnormal daughter, who had presented with a clinical picture consistent with 18q deletion syndrome. This parent-to-child “expansion” is likely due to a recombination mechanism.

Duplication

Duplicated segments may arise from within the same chromatid, from the sister chromatid, from the same arm, from the other arm, or from a different chromosome, through the similar mechanisms as described earlier. For a number of segments, both deletions and duplications have been recognized, and these cases are listed in Table 19–1. As with the deletion, the association of different duplicated segments with particular phenotypes offers an insight into which regions of the genome may harbor specific critical genes (Brewer et al., 1999). In general, identification of the phenotype-causing genes from duplications is more difficult than deletions, with partial trisomy typically having a lesser phenotypic consequence than partial monosomy. In some (by no means all) cases, the duplication (or sometimes triplication) of a locus manifests as the “opposite” phenotype as that found in the deletion. Thus, triplication of the *MMP23* gene on 1p36 results in craniosynostosis, while the deletion is associated with a large, late-closing anterior fontanel (Gajecka et al., 2005). Deletion of the *P* gene in the Prader-Willi/Angelman region causes lighter complexion (and when mutated causes oculocutaneous albinism type 2); an increased copy number of this gene leads to hyperpigmentation (Akahoshi, 2004). Shinawi et al. (2010) refer to these opposite scenarios as “sister genomic disorders” (and see later, dup 16p11.2).

Table 19–1. Some Chromosomal Syndromes for Which Both Deletion and Duplication of the Same Microsegment Have Been Described

	del	**dup**
1q21.1	del	dup
1q44	del	dup
2q37	Albright-like	dup*
3q29	del	dup
4p16.3	Wolf-Hirschhorn	dup
5p13	Cri du chat	dup
5q35	Sotos	dup
7q11.2	Williams	dup
10q22q23	del	dup
11p11.2	Potocki-Shaffer	dup
11p13	WAGR	dup
14q12	Congenital Rett	dup
15q11-q13	Prader-Willi, Angelman	dup
15q13.3	del*	dup*
15q24	del	dup**
16p11.2	del	dup
16p13.11	del	dup*
16p13.3	Rubinstein-Taybi	dup
17p11.2	Smith-Magenis	Potocki-Lupski
17p12	HPSN	CMT
17p13	Miller-Dieker	dup
17q12	del	dup
17q21.31	del	dup
20p12	Alagille	dup
22q11	diGeorge	dup
22q11.21-q11.23	del	dup*

Notes: Some syndromes have an eponymous nomenclature; most are noted simply on the basis of *del* or *dup* of the involved segment. Of these, Charcot-Marie-Tooth neuropathy (late nineteenth century), Angelman, cri du chat, diGeorge, Prader-Willi, Sotos, Rubinstein-Taybi and Williams syndromes, and pressure-sensitive neuropathy (mid-twentieth century), and Alagille (later twentieth century) are the only ones to have been described well in advance of the recognition of their chromosomal/genetic basis ("phenotype-first").

* Phenotype, in some at least, may have been considered to lie within a normal range.

** Single case, inherited from normal father; causal link uncertain (Kiholm Lund et al., 2008).

CMT, Charcot-Marie-Tooth neuropathy; HPSN, hereditary pressure-sensitive neuropathy.

Source: All entries from published reports, other than for dup 11p11.2, which is a personal communication from J. M. Gastier-Foster (2009).

Direct and Inverted Intrachromosomal (Tandem) Duplication.

The duplication comprises chromatin of the same chromosome, the original and the duplicated segments being ordered in tandem fashion. If the linear orientation of a chromosome A is maintained, the rearrangement is a direct duplication, 46,dir dup(A); if it is reversed, it is an inverted duplication, 46,inv dup(A). As noted earlier, many

apparent inverted duplications may in fact exist along with a terminal deletion; the causative mechanism may be either to NAHR or NHEJ, and most often occurring at a premeiotic mitosis (Rowe et al., 2009; Zuffardi et al., 2009). The inv dup/del 8p is a well-recognized recurring example (Kotzot et al., 2000b; Kondoh et al., 2003). An alternative explanation, based on an analysis of many cases of complex rearrangements of 1p36, is that successive breakage-fusion-bridge cycles result in deletion/duplication chromosomes, particularly near to the telomeres (Ballif et al., 2003b). Faivre et al. (2000b) propose a postzygotic sister chromatid exchange in a child with a condition resembling Sotos syndrome and having mosaicism for a dup(20)(p11.2-p12.1) in 23% of cells. On checking DNA markers that mapped to the region of the duplication in 20p, each parent had contributed just one allele. The parsimonious explanation is that a single event of unequal sister chromatid exchange happened at a mitosis, in an initially normal conceptus, generating the dup(20p) line, with the complementary deletion being lost (Fig. **19–3**).



Figure 19–3
A mechanism whereby a mosaic duplication and/or deletion could be produced. An unequal sister chromatid exchange at a postzygotic cell cycle generates a chromosome with a duplication (dup) in one chromatid and a deletion (del) in the other. At the next mitosis, the two chromatids segregate to the daughter cells, giving rise to a dup cell line and a del cell line. The del line may be lost, since partial monosomies are generally less survivable than partial trisomies, in which case only the cells of the dup lineage would exist alongside the normal cells. (After Faivre et al., 2000b.)

One rather subtle intrachromosomal duplication, and possibly triplication, needs to be distinguished from a normal variant. This is the “dup(8)(p23.1),” listed as a euchromatic variant in Chapter 16 (p. 264). On classical cytogenetics, the two forms are indistinguishable. But a replication of certain sequences within this region, different from those underlying the normal variant, can produce a phenotype due to a dosage effect of these segments (Barber et al., 2005). The phenotype may include minor cognitive impairment, mild facial dysmorphism, and structural heart defect, the latter possibly due to perturbation of the *GATA4* locus. Such cases typically arise de novo, and this is a clinical pointer enabling recognition of this pathogenic dup/trp.

Additional Material from Another Chromosome.

In other rearrangements, the duplicated material has come from another chromosome. An example of the karyotype nomenclature, to be used before the nature of the additional material has been established, is 46,add(1)(q36). Pairing between nonhomologs, followed by crossing-over (due to NAHR or NHEJ), produces reciprocal products that are two derivative chromosomes. In a “single-segment” exchange, one of these will have a duplication, and the other a deletion. If this occurred during meiosis, and if segregation were then asymmetric, gametes with a duplication or with a deficiency would be produced. Other scenarios, with more complex mechanisms, may be imagined. Coles et al. (1992), for example, studied a child with Wolf-Hirschhorn syndrome who had two separate de novo rearrangements of the X chromosome with a chromosome 4 and the Y, respectively, and they propose that simultaneous or sequential crossovers happened in a meiotic “octad” of four synapsing chromosomes.

If a de novo unbalanced rearrangement could be shown to have its component parts originating from a maternal and a paternal chromosome, the fact of its postzygotic origin would be thereby demonstrated. Sarri et al. (1997) offer an example of this scenario in a malformed child with 46,X,der(X), t(X;17)(q27;q22) whose der(X) originated from the paternal X and the maternal 17 chromosomes. Eggermann et al. (1997) report a similar case, an abnormal child with a de novo der(18)t(13;18)(q14.3;q23). The chromosome 18 component of the translocation came from the paternal chromosome 18, and the chromosome 13 component from the maternal chromosome 13. In this type of biparental rearrangement, even the very small theoretical risk otherwise associated with parental gonadal mosaicism could confidently be excluded.

Similarly, mosaicism in the presence of a normal cell line would typically allow the presumption of a mitotic origin. Zaslav et al. (1999) report a child with a severe brain malformation who had the karyotype 46,XX,der(4)t(4;15)(q35;q22)/46,XX. They propose that the chromosome constitution at conception was 46,XX. At an early cell division, a reciprocal exchange occurred between chromatids of chromosomes 4 and 15. Then, at anaphase, there was an unfortunate segregation. The newly generated der(4) passed to one daughter cell, along with the normal chromosome 15; and, vice versa, the der(15) and the chromosome 4 passed to the other. The former produced a cell line with a del(4)/dup(15) imbalance, and the presence of this cell line in the developing nervous system presumably caused the brain maldevelopment. The other cell line was not seen (on a peripheral blood karyotype), and it may have been selected against. (If the segregation of the chromosomes at that crucial mitosis had been balanced, then the child would likely have been a phenotypically normal mosaicism balanced translocation carrier.) Reddy and Mak (2001) could demonstrate mosaicism in both blood (conventional karyotyping) and on buccal mucosal cells (FISH) in two patients with additional material from another chromosome. For example, one patient had mosaicism for an add(5), the additional material coming from 3p26-pter, in 32% of lymphocytes. Using a 3p-subtelomere probe, a very similar level of mosaicism (40%) was shown in buccal epithelial cells.

Triplication.

A very few cases are known, at the level of classical cytogenetic analysis, of a segment of chromosome replicating twice over, being in three-fold amount on that homolog. The segment is thus present, in total, in four-fold dose. Triplications observed on classic cytogenetics are reported for chromosomes 2, 4, 5, 7, 9, 10, 12, 13, 15, and 16; in the triplication 12p case, the phenotypically normal mother was a low-level (12%) mosaicism for the same rearrangement (Eckel et al., 2006). On microarray, we have seen several more cases of triplication, and it seems likely that this “rare” complexity may be less rare than initially supposed. Familial transmission is recorded: the 46,XX,trp(4)(q32.1q32.2) mother in Wang et al. (2009b) had three sons with the same imbalance, 46,XY,trp(4)(q32.1q32.2), this being clearly visible on classical karyotyping.

Supernumerary Marker Chromosome

Many different supernumerary marker chromosomes (SMCs) exist, in the general karyotype 47,+mar (*mar* for “marker”), or 47,+SMC; they are also known as extra structurally abnormal chromosomes (ESACs), and accessory chromosomes.[2] Small ones are abbreviated as sSMCs, and very small, minute ones as minSMCs. The 47,+SMC individual has a duplication (partial trisomy), or in some cases a triplication (partial tetrasomy) of the material comprising the SMC. The birth prevalence is in the range 2–7 per 10,000. Blennow et al. (1995) record a large Scandinavian experience: of 50 SMCs, almost half were idic(15), six were small rings deriving from various autosomes, six were

isochromosomes of 18p or 12p, and most of the remainder were harmless SMCs derived from acrocentric chromosomes. A particular category is the SMC that lacks α-satellite DNA (a component of the normal centromere) but which possesses a "neo-centromere," a point of considerable theoretical interest and possibly practical significance (see later section on "Rare Complexities").

With increasing sophistication of staining techniques, not to mention the advent of array-CGH,[3] the expression "SMC" is to be seen as a temporary designation, awaiting the full delineation of whatever partial trisomy it may be. Readers who delight in bold colors should refer to Reichenbach et al. (1999). These authors describe a child with 47,+mar who, upon multicolor banding, could be seen as having the karyotype 47,+del(5)(q11). Guanciali-Franchi et al. (2004) used spectral karyotyping to identify a collection of 14 previously cryptic SMCs. By contrast, an absence of color was, in a sense, even more dramatic in a mildly abnormal child studied by Mackie Ogilvie et al. (2001): he had a C-band negative SMC that failed to show hybridization with any whole chromosome paint and could not be identified using a range of other cytogenetic methods (it also lacked a conventional centromere). The origin of this SMC was thus quite baffling.

Liehr (2008) reviews the subject at length, and he has a Web site devoted to the SMC: http://www.med.uni-jena.de/fish/sSMC/00START.htm

Isochromosomes

The classical isochromosome is a "mirror-image" chromosome, with two identical arms either side of the centromere; a more complicated rearrangement is the isodicentric (or inverted duplication) chromosome. When present as a supernumerary chromosome, the classic isochromosome imposes a tetrasomic state for the chromosomal arm concerned. Recorded isochromosomes include i(5p), i(8p), i(9p), i(10p), i(12p) (Pallister-Killian syndrome),[4] i(18p), i(18q), i(20p), i(22q), and i(Xq) (not including the Robertsonian acrocentric isochromosomes, which are dealt with in Chapter 7, and the i(21q) in Chapter 18). Isochromosomes can arise by a variety of mechanisms and at diverse times and places. The most simple, and classical, mode of isochromosome formation is a misdivision at the centromere, also known as "centric fission" (Rivera and Cantú, 1986). It can be thought of as a horizontal rather than a vertical division (Fig. **19–4**). This gives rise, in a chromosome A, to short arm and long arm isochromosomes, an i(Ap) and an i(Aq).[5] More complex scenarios can be devised, such as the U-type exchange, with the chromatid of one arm of a chromosome "looping around" to join with its fellow.

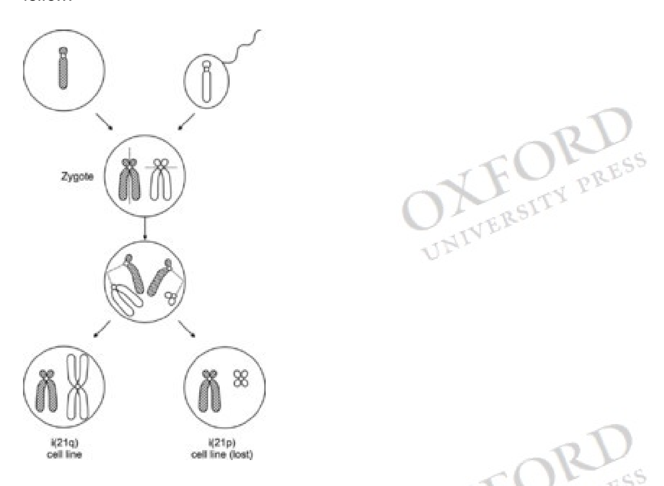


Figure 19–4
Outline of the classical theoretical mechanism to produce an isochromosome, by "horizontal misdivision" at the centromere. In this example, the site of its generation is at the first division of the zygote, considering the case in point of the i(21q) form of Down syndrome. The normal zygote has two chromosome 21 homologs (maternal crosshatched, paternal open). At the first mitosis, the maternal chromosome 21 divides appropriately at the centromere to give two normal daughter chromatids, but the paternal chromosome 21 misdivides. One product of the misdivision is an i(21q), and the cell resulting is trisomic for 21q (*lower left*). The other product is an i(21p), and this cell essentially has a 21 monosomy; its lineage does not survive. Thus, the child has a nonmosaic i(21q), and a typical DS phenotype. If the abnormal cell division occurs at a later mitosis (the second or subsequent), a mosaic 46,i(21q)/46,N karyotype could result.

Whatever the mechanism, the process could occur: in a premeiotic gametocyte (which would reflect a gonadal mosaicism); during one or other meiotic division; in the zygote; or at an early or a later postzygotic division, in an initially normal or an initially trisomic conceptus. The isodicentric chromosome, when present as a supernumerary chromosome, 47, +iso, typically arises following aberrant homologous recombination in meiosis. Subsequent mitotic loss of an isochromosome in one cell lineage can bring about a mosaic state, potentially having a less severe functional imbalance, which is not necessarily a fortunate event, since an otherwise early in utero lethal imbalance could convert to a survivable but profoundly abnormal phenotypic state. An interesting sequence of events is proposed in de Ravel et al. (2004), concerning the case of an abnormal fetus karyotyping 47,XX,+12/47,XX,+i(12)(p10) post termination. Initially, a disomic 12 egg met a monosomic 12 sperm, producing a trisomy 12 zygote. From one cell, a 47,+i(12p) mosaic line was created, due to rearrangement of the paternal chromosome 12 (and a normal cell line was also produced, seen only in short-term CVS culture). A similar scenario, with the postzygotic separation of a normal and an isochromosomal cell line, may have been the basis of karyotypically discordant, presumed monozygous twin fetuses, one with 47,XY,+i(5p), and the other 46,XY (Grams et al., 2011). In contrast, the isochromosome replacing a normal homolog, 46,iso, is almost always postzygotic in origin, from an initially normal conceptus, as for example Riegel et al. (2006) showed in two babies with Down syndrome and Patau syndrome. A normal karyotype had been seen at CVS, but the babies subsequently born karyotyped 46,i(21q) and 46,i(13q), respectively.

The very rare circumstance of recurrence of an isochromosome in siblings presumably reflects a premeiotic generation of the abnormality in a parental gonad (Krüger et al., 1987). The error in this circumstance could have occurred at mitosis in the parent, some distance along in their embryonic development, with only a small fraction of the body (minimally, a part of one gonad) involved. Or the error could have been present at the parent's conception, with a postzygotic loss of the supernumerary isochromosome in one cell line which becomes 46,N, and favorable distribution of this line in the soma, but not gonad, thereafter. Boyle et al. (2001) propose such an evolution in a family in which a mother, 46,XX on blood karyotype, had had two children (half-sisters) with nonmosaic 47,XX,i(18p). She herself, therefore, must have been a gonadal mosaic. As these workers show, it is likely that she had been 47,XX,i(18p) at her own conception, due either to a meiosis II error or a premeiotic mitotic error in her own mother's oögenesis. In her postzygotic development, the i(18p) line was lost in most tissue, but not in gonad. And surely, one would have thought, gonadal mosaicism was the reason for the father in Williams et al. (2001) having had one child with i(18p) and another with i(18q). A centromere misdivision in an early gametic stem cell mitosis was the obvious explanation. But on sperm analysis, over 1000 cells counted had just a single 18p and a single 18q signal. Examples like these oblige some caution in counseling, although it is true that such cases are very rare.

Isodicentric 15.

A supernumerary marker chromosome that warrants special attention is the bisatellited dicentric marker, "idic(15)," also known as inverted duplication 15, inv dup(15), or pseudodicentric 15 (Battaglia, 2008; Wang et al., 2008). The usual mechanism of formation is a translocation event between homologs during maternal meiosis, due to apposition of low copy repeats that are located within 15q11-q14. After formation, there is a nondisjunction event and centromere inactivation.

Depending on the sites of recombination, there are several types of inv dup(15), classified according to the amount of euchromatic material present. Generally, they are bisatellited and pseudodicentric (Wandstrat and Schwartz, 2000). The important clinical question is this: Is the Prader-Willi and Angelman critical region within 15q12–13 (PWSCR/ ASCR) included in the chromosome? Probes for *D15S10* and *SNRPN* targeting the PWSCR/ASCR are useful in assessing the amount of material (Eggermann et al., 2002). The markers can be divided into three groups:

(1) Very small chromosomes with so little chromatin between the centromeres that the appearance is monocentric, and FISH-negative for the 15q12 imprinted region with respect to the loci *D15S10/SNRPN*. These marker chromosomes are assumed not to be causally associated with an abnormal phenotype, and many times they are inherited from a clinically normal parent. Possibly, there may be an association with infertility in the male (Oracova et al., 2009).
(2) Medium-size chromosomes with two distinct centromeres with visible intervening chromatin. Because it is difficult to distinguish these from the next group, FISH with *D15S10/SNRPN* should be performed to be sure that the PWSCR/ASCR segment is not present. An inv dup(15) lacking this segment is typically not associated with an abnormal phenotype.
(3) Medium and larger (greater in size than a G-group chromosome) chromosomes, and which stain positively for *D15S10/SNRPN*, and which thus include the PWSCR/ASCR region. The presence in trisomic or tetrasomic dosage[6] of the PWSCR/ASCR segment—and in particular when of maternal origin—correlates with abnormality, and the phenotype encompasses pervasive developmental disorder with autism, epilepsy, and minor physical defects (Battaglia, 2008).

Thus, a reasonable approach to sorting out the various types of inv dup(15q) marker chromosomes, after identification on G-banded analysis, includes FISH for the 15q12 imprinted region (*D15S10/ SNRPN*) on metaphase chromosomes. If the region is present, interphase FISH can be used to examine whether there is one or two copies. If it is unclear whether the marker is of 15 origin, one might choose to go directly to microarray analysis; indeed, the initial recognition may have been based upon this methodology. This would also enable an assessment of the amount of euchromatin involved, albeit that there is not necessarily a strong correlation between the copy number quantum of some imprinted loci and the clinical phenotype (Hogart et al., 2009). Another caveat is that microarray analysis may not detect low-level mosaicism.

Isodicentric 22: Cat-Eye Syndrome.

One of the better known SMCs is the inv dup(22)(pter-q11.2) of the "cat-eye syndrome," and Mears et al. (1994) and Rosias et al. (2001) provide reviews. Two loci *CECR1* and *CECR2* may be critical dosage-sensitive genes (McDermid and Morrow, 2002). The region that is duplicated can vary, in some cases extending to include the region of the dup22q11 syndrome (Yobb et al., 2005). The chromosome is not necessarily symmetrical (not truly "iso"), depending upon the actual sites of homologous recombination within 22q11 that led to the rearrangement, and the euchromatic region may be present in trisomic or tetrasomic state. Most cases arise de novo, but familial transmission is recorded, including, remarkably, familial mosaicism (Urioste et al., 1994b). The phenotype appears not to correlate well with the size of the chromosome and indeed the person may show no signs of the syndrome (Crolla et al., 1997); the characteristic heart defect is total anomalous pulmonary venous return. Bergman and Blennow (2000) describe the unique case of a phenotypically normal man with inv dup(22) mosaicism, who also had a 22q11 deletion and a ring 22; it is plausible that the three abnormal chromosomes arose from related recombinations.

Rare Complexities

Supernumerary Marker Chromosome with Neocentromere.

Most neocentromere-SMCs exist as an inverted duplication, with the neocentromere forming on one of the two otherwise identical "arms" (rather than at the inv dup breakpoint). The two "arms" add up to a tetrasomic state (Voullaire et al., 2001; Mascarenhas et al., 2008). These neocentromeres lack α-satellite DNA and its centromere binding protein (CENPB) (hence the alternative name "analphoid centromere"). In other respects, neocentromeres function similarly to normal centromeres as evidenced by mitotic stability (in many, but not all cases) and by the binding of other known centromere proteins, such as CENPA, CENPC, and CENPE (Saffery et al., 2000; Voullaire et al., 2001). Most chromosomes are now represented in the list of those in which a neocentromere has been identified. Neocentromerization can be considered as a process based upon an epigenetic mechanism: the DNA in this region is the same as in the normal chromosome, but it has now been influenced to take upon itself a new identity and function (Amor and Choo, 2002). More complexities, and including the suggestion of a role for the neocentromere in evolution, are addressed in Amor et al. (2004); an example of a neocentromeric chromosome providing balance for a corresponding deletion is noted later (del 11p11.2).

Centromere-Telomere Fusion.

Centromere-telomere fusion is a rare mechanism to form an isochromosome. So far, this has been observed with an isochromosome for the short arm, and the long arm of that chromosome being attached to the telomere of another. Rivera et al. (1999b), for example, describe an iso(12p), in which the 12q element was translocated to 8pter. They could show FISH signals for both centromere and telomere probes at the 8pter/12cen union point in the translocation chromosome. The probable mechanism is a postzygotic centric fission of the 12, with the 12q element combining through a centromere-telomere fusion, and with the 12p element doubling up to produce the isochromosome. The end result was trisomy for 12p. Besides 12p, this scenario has been observed in isochromosomes for 4p, 5p, 7p, 9p, and 10p. All cases have been de novo.

Complementary Isochromosomes.

This very rare circumstance is dealt with in Chapter 8.

Apparently Balanced But Actually Unbalanced Rearrangement

If, on classical cytogenetics, an apparently balanced de novo translocation, inversion, or insertion is discovered in a patient with a "chromosomal" phenotype of dysmorphism/anatomical abnormality and developmental delay (young child) or cognitive compromise (older child, adult), it is logical, and sometimes correct, to assume a causal link (Tonk et al., 2003). Further cytogenetic analysis may reveal that there is, in fact, an actual imbalance.

With the advent of array-CGH, a clearer light, and often greatly clearer, can be cast upon these cases (Gajecka et al., 2006; De Gregori et al., 2007; Baptista et al., 2008; Sismani et al., 2008). Loss or gain of DNA at the site(s) of rearrangement provides strong supporting evidence in favor of a pathogenic effect, and particularly so if specific genes can be implicated. Conversely, a normal quantitative result would point to the rearrangement being truly balanced, although the possibility of disruption of a gene at the actual site of a breakpoint could yet be the explanation for an abnormal clinical picture (Cacciagli et al., 2010). Occasionally, the result is surprising: there may be no DNA imbalance at the site of breakpoints, but a deletion and/or duplication is seen at some other place in the genome. Here, the rearrangement can presumably be exonerated, as a coincidental event; the real cause lies elsewhere. Note that if the rearrangement that disrupts the gene is a "clean" break, with little or no loss of DNA, then a microarray would not detect it.

Position effect is another mechanism whereby a "balanced" rearrangement can lead to phenotypic abnormality. The *SOX9* gene on chromosome 17 at band q25.1, the basis of campomelic syndrome, provides an example with respect both to a translocation and an inversion. The de novo translocation t(5;17)(q15;q25.1) in Figure **19–5** was seen in a child with this syndrome, as was the de novo paracentric inversion inv(17)(q24.3q25.1) reported in Maraia et al. (1991). Other such examples are mentioned later (see del 7q21.3, del 11p11.3). This type of rearrangement would probably not be detectable on microarray.

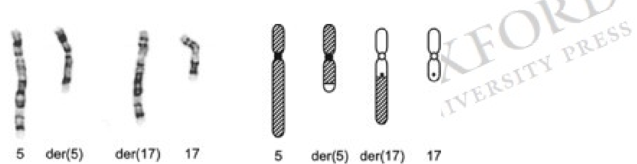


Figure 19–5
An apparently balanced translocation causing the syndrome of campomelic dysplasia (which includes skeletal, genital, and brain defects). One breakpoint is at 17q25.1, on or close to the *SOX9* locus (shown as dot on the cartoon karyotype), where the basis of the syndrome lies. One possibility is that the gene is disrupted. Or, an influence of adjacent chromosome 5 chromatin ("position effect") leads to inactivation of the *SOX9* gene on the der(17), the functional *SOX9* haplo-insufficiency then being responsible for the phenotype. (Case of R Savarirayan; Savarirayan and Bankier, 1998.)

Genetic Counseling

Deletions and Duplications

In most children with deletions or duplications, the parents type as 46,XX and 46,XY on routine blood analysis, and the defect is "de novo." The risk for recurrence is very small, but it is not nonexistent. An example is noted later under "del 5p"; recurrence of a typical Prader-Willi deletion in brothers is recorded in Fernández-Novoa et al. (2001); and a del(9)(q12q21.32) has been seen in siblings (M. D. Pertile, personal communication, 2010). Röthlisberger and Kotzot (2007) undertook a review and were surprised at how few actual cases had been published. The rare recurrences are likely due to an occult parental mosaicism, which the routine blood chromosome study could not detect. The abnormal line in the parent may be gonadal (confined to gametic tissue) or somatic-gonadal (some somatic tissues involved as well—but not, apparently, blood). The observation of rarity of recurrence allows us to propose the empiric advice that, in the individual case, recurrence is most unlikely. A figure that is appropriate in this setting might be "less than ½%"; the counselor should note the converse "greater than 99½%" for a child without the chromosome defect. Even this may be an overstatement: Röthlisberger and Kotzot (2007) consider that "less than 0.3%" may be closer to the mark. Some couples may find "greater than 99.7%" sufficiently encouraging that they would not request prenatal diagnosis in a subsequent pregnancy. Some, however, might; and one could sympathize with this request. If testing is to be based upon classical cytogenetics alone, care must be exercised in offering prenatal diagnosis of small deletions/duplications seen only on high-resolution lymphocyte chromosomes. The technical ability to demonstrate such small changes in amniotic fluid or chorionic villus cells may be limited.

In those deletions/duplications where a parent is shown to carry a balanced rearrangement, a substantial recurrence risk is probable, and the appropriate chapter should be consulted. Rarely, the same deletion/ duplication might be seen, somewhat unexpectedly, in a parent, an observation that underpins the advice that parental karyotyping does need to be undertaken (Sparkes et al., 2009). Extremely rarely, an inverted duplication may, in fact, be due to recombination within a parental paracentric inversion (p. 181). Paracentric inversions can be difficult to detect, and a careful and directed search may be appropriate.

With the increasing use of powerful molecular cytogenetic methodologies, and with particular reference to array-CGH, many more cases of chromosomal imbalance are being recognized (Shaffer and Bejjani, 2006; Shaffer et al., 2007a; Bejjani and Shaffer, 2008; Slavotinek, 2008). Figure **19–6** lists recorded cases on the European Cytogeneticists Association database (see later). Documentation of such cases is of much value to the clinician, but the sheer volume of new discoveries does represent a challenge. One organ, the *European Journal of Medical Genetics*, is devoting an occasional section headed "Chromosomal Imbalance Letters," and this is likely to prove a useful repository, and likewise DECIPHER is a valuable source (see next section).

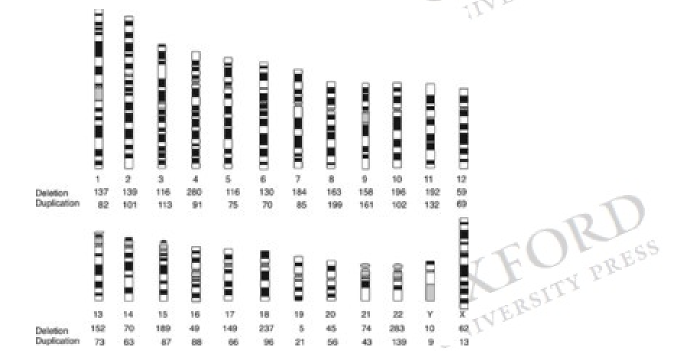




Figure 19–6
The listing of the numbers of deletions and duplications observed per individual chromosome, from the database of the European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (ECARUCA), as of July 2009 (http://www.ecaruca.net). (Reproduced with the permission of ECARUCA.)

Internet Resources.

A number of databases have been developed, accessible on the Internet, and these are a most valuable resource. We may list the following:

- The European Cytogeneticists Association, building substantially upon the work of the group of Professor Albert Schinzel in Zürich, has established a register of unbalanced chromosome aberrations (ECARUCA), which can be accessed—and contributed to—at http://www.ecaruca.net (Feenstra et al., 2006). A particular chromosomal abnormality can be searched for, according to its standard cytogenetic description, or by array-CGH criteria, at the level of the nucleotides encompassing a deletion or duplication. ECARUCA publishes from time to time their totals of deletions and duplications per chromosome, and the July 2009 listing is shown in Figure **19–6**.
- The UK Chromosome Abnormality Database (UKCAD) provides data from all the UK Regional Cytogenetics Centers and is accessed at http://www.ukcad.org.uk/cocoon/ukcad/
- DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources) is a repository of phenotypes for chromosome alterations identified by microarrays, and it can be accessed at http://decipher.sanger.ac.uk/
- Signature Genomic Laboratories have developed an in-house database, "Genoglyphix," of microarray-based chromosome diagnoses. This is open to the referring clinicians, laboratory diagnosticians, and genetic counselors, who can generate their own databases through this system. The Web site address is http://www.signaturegenomics.com/genoglyphix.html

Deletions

Brief sketches of the major deletion syndromes follow, as well as some less well-known ones, in numerical order of chromosomes, and numerical order of p and q segments. Some gain inclusion because of one specific and striking feature, such as the del(5) syndrome with polyposis. We comment in greater or lesser length upon the genetics of each. In some, we make mention of familial transmission; but primarily we are dealing with de novo defects. The better known ones are depicted in the composite karyotype in Figure **19–7**. In the limit, every different deletion, even if only one case is known, could be regarded as a "new syndrome." As Figure **19–6** attests, from the ECARUCA site, rather a large number of deletions, and an increasing number of duplications, are coming to be recognized. Chromosomal atlases and catalogs provide further clinical information, and the essays in Cassidy and Allanson's *Management of Genetic Syndromes* (2005) offer detailed commentaries for some of the more common of these

syndromes (Angelman, Prader-Willi, Russell-Silver, Smith-Magenis, velocardiofacial, and Williams syndromes).

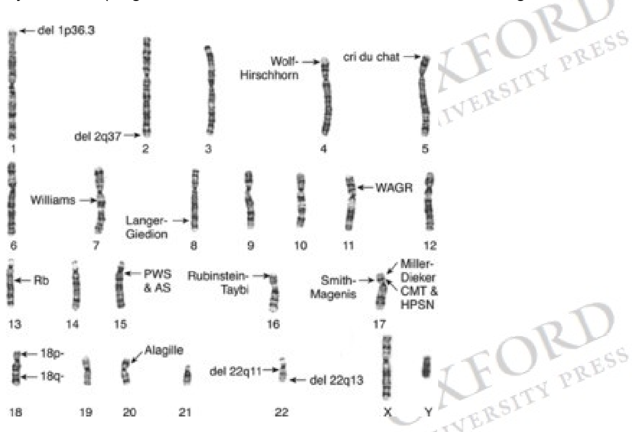




Figure 19–7

“Composite karyotype” showing the site of the cytogenetic defect in some of the deletion syndromes. AS, Angelman syndrome; CMT, Charcot-Marie-Tooth neuropathy (a duplication); HPSN, hereditary pressure-sensitive neuropathy; PWS, Prader-Willi syndrome, Rb, retinoblastoma plus other features; WAGR, Wilms tumor, aniridia, genital defects, retardation syndrome.

Chromosome 1.

del 1q21.1.

“New” syndromes, in which the clinical observations are rather nonspecific, or at least variable, are coming to be recognized, and especially as several groups in numerous countries pool their microarray experience. The microdeletion of chromosome 1q21.1 is such an example, and the clinical picture is described in a paper of which the co-authors number 84 (Mefford et al., 2008). Mental retardation, autism, seizures, cardiac defects, and cataract, are included. There may be a hint of a Williams-like facies. Some are de novo cases, some familial with a (usually mildly) affected parent, and some familial from an apparently unaffected parent. 1q21.1 deletion also has the interest of an association with thrombocytopenia-absent radius syndrome (p. 265). A larger deletion embraces a broader phenotype (Velinov and Dolzhanskaya, 2010).

del 1p36.3.

The del(1)(p36.3) syndrome may be of similar frequency to the del(22q) syndrome, with these two conditions being the most common syndromes of, respectively, terminal and interstitial deletion. The deletions can be of variable extent (Heilstedt et al., 2003). The clinical features of this syndrome are reviewed in Gajecka et al. (2007) and Battaglia et al. (2008). The facies is variably dysmorphic, and several minor physical anomalies may be observed. The mental defect is usually severe; an unsurprising observation, given that a major brain malformation, perisylvian polymicrogyria, is a characteristic neuroradiological correlate (Dobyns et al., 2008).

The segment 1p36.3 is a light-staining region, and thus the deletions, which can be of variable size, are not always visible on light microscopy. In three studies of “karyotypically normal” retarded and dysmorphic populations using subtelomeric FISH, two del(1)(p36.3) cases were identified among a total of 411 individuals tested (Anderlid et al., 2002; Baker et al., 2002; Clarkson et al., 2002). Using an initial molecular approach (a panel of microsatellite markers to pick up 1q36.3 hemizygosity), Giraudeau et al. (2001) screened 567 patients with mental retardation and found three with del(1p), in whom FISH confirmed the deletion. These combined data suggest that about 0.5% of this category of population, in whom a standard karyotype has been interpreted as normal, may have this subtelomeric deletion, and nowadays most diagnoses are likely to be made through microarray. The majority of cases of del(1)(p36.3) syndrome arise de novo, but transmission from a parental translocation is recorded, as is presumed parental gonadal mosaicism (Shapira et al., 1997b; Gajecka et al., 2008b, 2010).

del 1q41q42.

This syndrome is another example of discovery by the “genotype-first” approach (Shaffer et al., 2007b). Because certain genes are recognized to cause developmental disabilities, it was presumed that genes within those pathways, not (as yet) shown to have effects on development, might also be candidates for genetic disease. A microarray was constructed that targeted these genes, and among these was the *DISP1* locus, a gene that directly regulates sonic-hedgehog (*SHH*). The SHH signaling pathway, when perturbed, can cause midline brain defects, including holoprosencephaly. Patients were identified with *DISP1* loss, and hence the del(1)(q41q42) basis of this loss. Although these patients do not have holoprosencephaly, they exhibit midline defects, have severe mental retardation, and a notable dysmorphology and malformation pattern, including diaphragmatic hernia, reminiscent of Fryns syndrome[7] (Kantarci and Donahoe, 2007).

Chromosome 2.

del 2q23.1.

This syndrome, typically de novo, has the interest of a resemblance to Angelman and Rett syndromes, with features of severe mental retardation with absent speech, stereotypic repetitive behavior, microcephaly, ataxia, seizures, and a coarse facies. The crucial genes may be *MBD5*, a member of the same family as the *MECP2* gene of Rett syndrome, and *SATB2*, these genes both having the capacity to influence expression of multiple other target genes (Jaillard et al., 2009; Rosenfeld et al., 2009; van Bon et al., 2010; Balasubramanian et al., 2011).

del 2q37: Albright-like Syndrome.

This cytogenetic defect should specifically be sought in patients with a morphological phenotype somewhat reminiscent of Albright hereditary osteodystrophy (short stature, short metacarpals), a quite distinctive facies, and intellectual deficit. It may be among the more frequent of the deletion syndromes, the subtlety of the cytogenetic defect having previously obscured its role (Phelan et al., 1995; Wilson et al., 1995). Deletions of lesser extent, detectable only upon molecular methodologies, may display substantially the same clinical phenotype (Kitsiou-Tzeli et al., 2007). Parent-to-child transmission is very rare, but has been observed. Familial translocations have been reported (Batstone et al., 2003).

Chromosome 3.

del 3p26.2.

Differing degrees of distal 3p deletion are associated with differing phenotypes, although a core contributor to the mental retardation is likely to be loss of the gene *SRGAP3* (Shuib et al., 2009). Two genes within most 3p deletions are *VHL* and *ITPR1*, haplo-insufficiency of which cause Von Hippel-Lindau disease, and spinocerebellar ataxia type

15, respectively (Van de Leemput et al., 2007). One would thus predict that a del(3p) patient whose deletion included these genes, and surviving well into adulthood, might develop each of these tumor-associated and ataxic syndromes.

del 3q29.

Patients with variable extents of 3q29 deletion, approximately 1.6 Mb in size, have been described by Willatt et al. (2005) and Ballif et al. (2008). Mild-to-moderate mental retardation with microcephaly, and fairly subtle facial dysmorphism, are core features. Some have, in addition, had clefting, and genitourinary malformation. Familial transmission is recorded (Ballif et al., 2008; Li et al., 2009; Digilio et al., 2009). The deletion is flanked by segmental duplications, and likely arises from nonallelic homologous recombination, and thus it is not surprising that the reciprocal duplication is observed (see later, dup 3q29).

Chromosome 4.

del 4p: Wolf-Hirschhorn Syndrome.

This well-known deletion syndrome identified in the prebanding era is one of the few that can, in its typical form, be confidently recognized clinically. The natural history of Wolf-Hirschhorn syndrome (WHS) is discussed in Battaglia and Carey (1999). Classical deletions are detected on routine cytogenetics, whereas molecular approaches are needed to identify subtler deletions (Maas et al., 2008; Galasso et al., 2010). Zollino et al. (2008) propose that the condition be considered in two typical forms: the more common classical form, and a relatively mild form, and in addition a severe form not resembling classical WHS, and these several forms correlate with the extent of the deletion. Maas et al. discuss the genotype-phenotype correlations, with respect to the precise extent of the deleted segment (as defined by array-CGH), and propose genes whose haplo-insufficiency may be the basis of particular traits. While most WHS occurs de novo, almost half may, upon further analysis with array-CGH, be revealed as having arisen as unbalanced translocations, and they may show additional phenotypic features in consequence (South et al., 2008b; Galasso et al., 2010).

The improvement in precision of cytogenetic and latterly the development of microarray methodology has enabled the elucidation of some malformation syndromes previously supposed to have been due to autosomal recessive inheritance. The Pitt-Rogers-Danks syndrome turned out to be the less severe phenotypic version of WHS, as noted earlier. A family with the supposed recessive Lambotte syndrome of multiple malformation, having surprisingly produced a further affected child in a succeeding generation, was subjected to detailed FISH analysis, and a familial t(2;4)(q37.1; p16.2) identified (Herens et al., 1997). Here, the phenotype in fact reflected the combined effects of partial 4p monosomy and 2q trisomy. A syndrome of mental retardation with polymicrogyria originally reported as being X-linked transpired as being due to an autosomal translocation, t(1;12)(q44;p13.3), with both adjacent-1 segregations being represented (Zollino et al., 2003). Other examples exist, and Verloes et al. (2000) speak of these as “pseudo-recessive disorders” being revealed in their true colors as due to cryptic translocations.

A deletion just centromeric of the WHS region, at 4p15.2-p15.32, leads to a quite different disorder, in some respects resembling Marfan syndrome (Basinko et al., 2008).

del 4q34.

It may be that among all the chromosomal disorders no single discrete physical sign is pathognomonic for a particular aneuploidy (that is, seen in this specific condition, and in no other). But there is one sign that might be: a duplicated nail of the fifth finger, with one nail in the normal position, and the other where the fingertip pad should be (thus, dorsal and volar surfaces). This curious observation, it is proposed, can enable the clinician to predict a distal 4q deletion, to be precise, of 4q34.2 (Vogt et al., 2006). A deletion just a little above, into 4q33, may be associated notably (although not pathognomonically) with abnormality of the ulnar ray of the upper limb (Keeling et al., 2001). The range of deletion and phenotype, and consideration also of duplication and more complex distal 4q rearrangement, is documented in Rossi et al. (2009).

Chromosome 5.

del 5p: Cri-du-Chat Syndrome.

The breakpoints in this famous syndrome are very variable: in a molecular study of 62 Italian cases, for example, at least 28 different sites were identified, from p13 through p15.2. The “*cri*” region is pinpointed to proximal p15.3, and certain other components of the phenotype can be attributed to certain segments within p14, p15.1, p15.2, and p15.3 (Fig. **19–8**) (Kjær and Niebuhr, 1999; Cerruti Mainardi et al., 2001). Kjær and Niebuhr suggest that anomalous formation of the notochord in the early embryo may then compromise the development of certain cranial nerve nuclei in the subjacent brainstem, affecting the innervation of the larynx, which is the actual anatomic structure that produces the “cat-like cry.” Deletion of the segment 5p15.3 alone can produce the typical cry in an otherwise normal child.

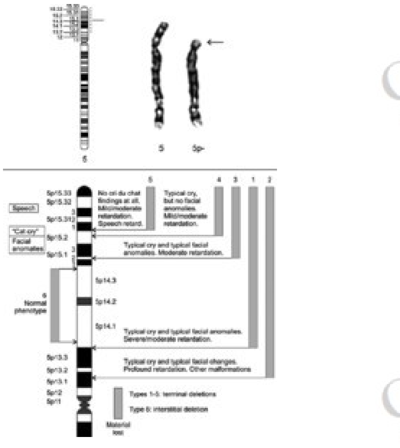






Figure 19–8
An example of the archetypal del(5p) chromosome (*above*), deletion of which is the basis of cri du chat syndrome, first described in 1963. A display of a detailed karyotype-phenotype correlation is shown below. Regions which may be implicated in speech, the characteristic cry, and facial anomalies are indicated at left. The arrows indicate five different breakpoints defining five different extents of terminal deletion, the lengths of which are reflected in the vertical bars to the right (numbered 1–5), and the associated phenotypes noted alongside. The vertical bar to the left (numbered 6) identifies an interstitial segment, deletion of which does not cause phenotypic abnormality. (From I. Kjær and J. Niebuhr, 1999, Studies of the cranial base in 23 patients with cri-du-chat syndrome suggest a cranial developmental field involved in the condition, *American Journal of Medical Genetics* 82:6–14. Courtesy I. Kjær and J. Niebuhr; reproduced with the permission of Wiley-Liss.)

Van Buggenhout et al. (2000) document in quite some detail, with several photographs, the phenotypes in seven older individuals, teenagers and adults. All but one had severe or profound mental defect. Perhaps surprisingly, the neurodevelopmental compromise does not correlate well with the size of the deletion (Marinescu et al., 1999a). Growth charts have been compiled from international data from 374 cases, from birth to age 18 years, and these document a substantial downward shift in the graphs for the three major indices (weight, height, head circumference) (Marinescu et al., 2000).

While most cases are sporadic, familial transmission from a parental translocation is recorded (Cotter and Musci, 2001), and this possibility should be checked for in each case. One case of recurrence of del(5)(p15.2), identified at prenatal diagnosis, attests to the reality of gonadal mosaicism (Hajianpour et al., 1991).

del 5p13.1: Cornelia de Lange Syndrome.

Chromosome 3 had initially been implicated in the cause of Cornelia de Lange syndrome (CdLS), but the true basis was revealed when two affected infants were identified with a rearrangement involving 5p13.1. A stillborn infant had a severe form of the condition, amniocentesis in the pregnancy having identified a 5p deletion, 46,XY,del(5)(p13.1p14.2); and another CdLS infant had a translocation t(5;13)(p13.1;q12.1). These cases enabled discovery that the gene causing CdLS is *NIPBL*—in the great majority due to a point mutation—located at 5p13.1 (Hulinsky et al., 2005; Selicorni et al., 2007). Recurrence due to paternal gonadal mosaicism for a *NIPBL* mutation is recorded (Niu et al., 2006). Suspected cases testing mutation-negative would prudently proceed to FISH or microarray analysis, to check for whole-gene deletion.

del 5q22-q23: Polyposis Plus Syndrome

A minor degree of facial dysmorphism and mild to moderate mental retardation are nonspecific features seen in deletions in the region of 5q22-q23; the unique feature is adenomatous polyposis of the bowel, and indeed it was such a deletion that led to discovery of the *APC* (adenomatous polyposis coli) gene (Hockey et al., 1989; Kobayashi et al., 1991). Absence of one *APC* allele of itself allows polyps to develop, and any subsequent mutation/loss of the allele on the intact chromosome 5 then leads to loss of the tumor suppressor function of this gene (Hodgson et al., 1994).

At the time of initial diagnosis in a child, a cancer risk could not have been anticipated. This exemplifies the point that parents need to be aware that, when a test procedure (microarray) is applied that can interrogate the whole genome, more information may come to light than they had been expecting. Nevertheless, although advice about a cancer risk may come as an "unwanted surprise," discovery of this deletion (and some others, as noted later) may in fact be lifesaving (Heald et al., 2007).

> Other similar examples exist of constitutional deletions that convey, in addition to congenital abnormality, a cancer predisposition. As with the polyposis example, the typical scenario is that loss of one allele of a tumor suppressor gene on the deleted chromosome comprises the "first hit" in the process of tumorigenesis. The 13q14 deletion associated with retinoblastoma, noted later, is the classic example. Jacoby et al. (1997) describe a deletion of 10q22.3-q24.1 in a patient with multiple congenital malformations and juvenile polyposis, the latter presumably reflecting the loss of one copy of the *PTEN* gene. A 9q22.32-q31.1 deletion has been associated with a "Gorlin syndrome plus" phenotype, and an increased risk for cancer is to be expected (see later section on "del 9q22.32q31.1"). We refer on p. 16 to the inadvertent discovery of a p53 deletion, predisposing to Li-Fraumeni syndrome. Carcinogenesis may also be due to gene amplification, and constitutional gain of a particular gene may thus contribute a risk. Willatt et al. (2001b) and Seven et al. (2002) draw attention to the link between constitutional 2p23 duplication and consequent duplication of the oncogene *N-myc*, and neuroblastoma. We note on p. 16 the risk for leukemia in trisomy 8 mosaicism.

del 5q35: Sotos Syndrome.

Most Sotos syndrome (cerebral gigantism) is seen with a normal karyotype. A few patients have had a chromosomal abnormality, and since two of these involved translocations with one of the breakpoints at 5q35, and since also some del(5)(q35) cases have had a Sotos-like phenotype, this had suggested there might be a Sotos locus in the region. This hypothesis was subsequently vindicated, the gene in fact being *NSD1* (Kurotaki et al., 2002). Most patients have a 2.2 Mb deletion, which encompasses *NSD1*,[8] with nonallelic homologous recombination due to *Alu* repeats the typical basis of this (Mochizuki et al., 2008).

Chromosome 6.

del 6q.

Three different segments of 6q have been associated with particular sets of clinical traits: del(6)(q11-q16), del(6)(q15-q25), and del(6)(q25-qter). Naturally, with these segments being genetically different, and only on the same chromosome by "evolutionary accident," the three phenotypic patterns are different, with mental retardation noted as the only universal finding (Hopkin et al., 1997). With respect to the q11-q16 deletions, molecular cytogenetics has since enabled a more precise understanding. Bonaglia et al. (2008) dissect out the candidate genes in deletions at 6q16.2 associated with a phenotype resembling Prader-Willi syndrome. Moving just 5 Mb centromerically, to 6q16.1, Traylor et al. (2009) report a child whose 2.16 Mb deletion removed only two known genes, *EPHA7* (ephrin receptor 7) and *TSG1*. The reasonable conclusion is drawn, in this latter case, that haplo-insufficiency for *EPHA7*, a gene whose expression field in the mouse equivalent includes neuronal synapses, has a key role in the functional neurological phenotype of the del(6)(q16.1) syndrome. Another specific deletion, in addition to those noted earlier, is del(6)(q23.2-q24.2), reported in just one case, and concerning a 3-year-old girl whose development was "completely normal to advanced," and who had been karyotyped as a newborn because of low birth weight (Kumar et al., 1999). While the facies was distinctive, she was said to resemble her family. One might imagine that this particular segment contains no critical brain loci.

Chromosome 7.

del 7p13: Greig Cephalosyndactyly Syndrome

This acrocephalopolysyndactyly syndrome, classically inherited as an autosomal dominant, is due to mutation at the *GLI3* locus. Rare microdeletion cases have haplo-insufficiency of *GLI3*, as well as loss of some adjacent loci, and the phenotype is combined Greig syndrome with neurodevelopmental defect, seizures, and other abnormalities (Kroisel et al., 2001).

del 7p21.1: Saethre-Chotzen Syndrome.

Most Saethre-Chotzen syndrome (a type of acrocephalosyndactyly) is due to point mutation in the *TWIST* gene at 7p21.1. Cytogenetic forms include microdeletions within this region, the larger of which remove other genes and add in a broader phenotype (Johnson et al., 1998; Touliatou et al., 2007; Busche et al., 2011). An apparently balanced translocation, which might disrupt the *TWIST* gene or perturb its function, is another mechanism. It appears that either mechanism, haplo-insufficiency or point mutation of *TWIST*, can lead to the similar phenotype. The skull defect in Saethre-Chotzen syndrome is premature fusion of cranial bones; it is interesting that a duplication ("triplo-excess") at this locus can produce the opposite effect, an underdevelopment of the cranial bones.

del 7q11.23: Williams[9] Syndrome.

Williams syndrome (WS) is due to a ~1.5 Mb deletion within chromosome 7q, and can arise from either parent (Wang et al., 1999a). Sperm studies in control donors show similar frequencies of deletion and duplication for this segment, pointing to a likely NAHR mechanism (Molina et al., 2011). The deleted loci include *GTF2I* and *GTF2IRD1*, which may well be key contributors to the neurocognitive profile and craniofacial morphology (Antonell et al., 2010; Ferrero et al., 2010), and the elastin gene, which is responsible for the cardiovascular component of the phenotype. The characteristic psychological phenotype is that of a mild intellectual disability, with overfriendliness to strangers, and a lacking in social judgment; an abnormally developed amygdala (a brain structure subserving social behavior and the recognition of emotional facial expressions) may be the basis of these traits (Martens et al., 2009). Earlier impressions that aspects of language might be intact have been refuted (Donnai and Karmiloff-Smith, 2000). Growth indices (height, weight, head circumference) for deletion-proven WS have been compiled (Martin et al., 2007b). Affected monozygous twins generally have a rather similar phenotype (Castorina et al., 1997). We know of no record of recurrence in siblings of undoubted WS to normal parents. Rare instances of parent to child transmission are known (Pankau et al., 2001).

Atypical cases with larger deletions may show a more severe phenotype, and those with smaller, a less severe. Thus, a child with a large deletion at 7q11.2 had components of WS, along with other features, including a severe mental defect (Wu et al., 1999); and a child with a smaller deletion showed lesser physical traits, and had an IQ within the

normal range (Ferrero et al., 2010).

del 7q21.3: Ectrodactyly Plus Syndrome.

One type of split hand and split foot malformation is associated with deletions in 7q21, and loss of one allele at a “digit-formation locus” in this region may be the basis, and candidate genes have been proposed (Basel et al., 2006). Loss of contiguous genes encompassed by the deletion may contribute to other less specific dysmorphology and to a diminished intellectual function (Roberts et al., 1991). An inversion in this region might have its influence due to “position effect,” with loss of expression of these putative gene(s), rather than haplo-insufficiency (van Silfhout et al., 2009). Other split hand/foot loci may reside in 2q14.1-q14.2, 4q32-q35, 5q15, and 6q16-q22 (David et al., 2009; Niedrist et al., 2009).

del 7q32-qter: Holoprosencephaly Plus Syndrome.

Holoprosencephaly is a developmental brain defect that can vary from devastatingly severe to rather mild, and there are several different genetic causes. Chromosomes 7 and 13 are important contributors. Distal 7q deletions were instrumental in the mapping of one locus, *HPE3* (Frints et al., 1998), that was narrowed to find the causative gene, *SHH*. De novo deletion is the rule, but familial holoprosencephaly has been recorded in the setting of a familial 7q36 translocation (Hatziioannou et al., 1991). Deletion 7q holoprosencephaly is due to *SHH* haplo-insufficiency. Another deletional cause of holoprosencephaly resides in chromosome 13, del(13)(q32), and reflects haplo-insufficiency for the *ZIC2* brain morphogenesis gene (Quélin et al., 2009). (This is in contrast to the holoprosencephaly associated with chromosome 13 duplication, as in standard trisomy 13.) In a series of holoprosencephaly patients in whom array-CGH was applied, Bendavid et al. (2009) identified a surprisingly large fraction with de novo microrearrangements, and they proposed that further brain genes might reside in these several regions.

Chromosome 8.

del 8p23.1-pter, or del 8p23.1.

Small terminal deletions of 8p are quite frequently recognized. A notable aspect of the phenotype is a severe behavioral disturbance in childhood, on the background of a mild mental defect. Sudden and extreme changes in behavior are observed, with outbursts of aggressiveness and destructiveness. Frustration tolerance is very low. Behavior seems to improve in later adolescence. The deletion may remove the gene for a cardiac transcription factor, *GATA4*, and this may be the basis of the observation that heart defects are frequent (Pehlivan et al., 1999). Diaphragmatic hernia may be a characteristic component (Shimokawa et al., 2004).

del 8q12: CHARGE Syndrome.

Most cases of this syndrome (coloboma, heart, choanal atresia, retarded growth and development, genital, ear defects) are due to mutation of the *CDH7* gene, with functional haplo-insufficiency, but in a few haplo-insufficiency is the consequence of a deletion in 8q12 that includes the *CDH7* locus (Vissers et al., 2004). More severe mental retardation in the deletion case may reflect the loss of adjacent “brain genes.” The deletion is typically de novo.

del 8q24.11-q24.13: Langer-Giedion Syndrome (Tricho-Rhino-Phalangeal Syndrome Type II).

The facies is distinctive, and diagnosis can be made with some confidence on clinical grounds. The condition is due to a deletion that removes the gene for tricho-rhino-phalangeal (TRP) syndrome type I, a bone growth control gene (*EXT1*, which causes exostoses[10]), and several other genes, to give the broader picture of Langer-Giedion syndrome (Lüdecke et al., 1999; Momeni et al., 2000). The deletion may arise on the chromosome 8 of either parent (Nardmann et al., 1997).

Chromosome 9.

del 9p22-p23.

Quite a number of 9p cases involving the p22-p23 region are recorded, over 100, and they present a characteristic phenotype. Many are due to deletions occurring in a region of about 5 Mb in 9p23: not so much a hotspot, but a series of hotspots. The deletion is equally likely to have happened on the paternal or maternal chromosome 9. Some that may at first sight seem to be simple deletions turn out, on FISH studies, to be due to other more complicated rearrangements (Christ et al., 1999; Swinkels et al., 2008).

del 9p24.3: Sex Reversal Plus.

This deletion syndrome is notable in having pointed the way to discovery of the *DMRT1* gene. This is the most conserved of any known sex-determining gene, and it is actually on the Z chromosome (the homogametic chromosome) of birds. Its expression is normally greater in the male than in the female embryo, and this dosage may be the basis of its testis-inducing action. It is proposed that the loss (or perturbation) of one *DMRT1* allele in (or adjacent to) the deleted segment brings the amount of product down below this threshold, and thus the 46,XY,del(9)(p24.3) person develops as a female (Calvari et al., 2000). An incomplete loss of function may lead to genital ambiguity. Loss of adjacent genes presumably contributes to the wider phenotype (Vinci et al., 2007).

del 9q22.32q31.1.

This deletion syndrome includes a susceptibility to cancer, due to involvement of the *PTCH1* gene, and thus the chromosomal diagnosis enables an informed surveillance to put in place (De Ravel et al., 2009).

del 9q34.3: Kleefstra Syndrome.

This subtelomeric microdeletion syndrome of severe intellectual deficit and multiple malformation has been observed in over 50 reported cases (Kleefstra et al., 2009) and may be, after 1q36 and 22q13, the third most frequent of the subtelomeric deletion syndromes. In the neonate, the facies may have a resemblance to Down syndrome. Haplo-insufficiency of the gene *EHMT1* is, of itself, the basis of the phenotype, and varying degrees of the extent of deletion appear not to influence the clinical picture. Analogous to Rubinstein-Taybi syndrome (see later), patients with classic mendelian mutations in this gene present essentially the same phenotype as do those with deletions. The protein encoded by to this gene, Eu-HMTase1, has a role in maintaining the integrity of histones that comprise a key component of the architecture of the chromosome; this syndrome can thus be considered as a disorder of chromatin remodeling. Parental somatic-gonadal mosaicism with recurrence in offspring has been recognized (Willemsen et al., 2011).

Chromosome 10.

del 10p13: DiGeorge Syndrome Phenocopy, and “HDR” Syndrome.

This deletion is the basis of a condition that may resemble DiGeorge syndrome (DGS; see later section on “del 22q11”), and indeed the condition has been labeled DGS2 (Van Esch et al., 1999), although not every such deletion will lead to a DGS-like clinical picture (Benetti et al., 2009). Some features of “DGS2,” such as ptosis and hearing loss, are not present in the 22q deletion form. It is clearly a rare cause of a DGS phenocopy, as no cases were found in a European study specifically addressing this question, and indeed the authors propose that searching for 10p microdeletions is not warranted in the service laboratory (Bartsch et al., 2003). However, in an American study, in which a dual-probe FISH for DGS1 and 2 was used, one case was found in 412 patients presenting with a possible diagnosis of DGS (54 had DGS1) (Berend et al., 2000b). Hypoparathyroidism is a frequent observation in del(10)(p13), but this is in fact due to haplo-insufficiency for the nearby *GATA3* gene, determining the HDR (hypoparathyroidism, sensorineural deafness, renal dysplasia) syndrome, when the deletion extends to include this locus (Fujimoto et al., 1999; van Esch et al., 2000; Benetti et al., 2009). Most HDR syndrome occurs sporadically, but inheritance due to parental translocation has been observed.

del 10q11.2: Hirschsprung Disease Plus Syndrome.

The locus for the receptor kinase gene *RET* is at 10q11.2 (the Hirschsprung chromosome region 1, HSCR1). In the haplo-insufficient state, certain neurons/neural crest cells may fail to migrate to their proper place and/or fail to undergo proper neuronal maturation in the intestinal wall. Without this nervous control, the segment of bowel is chronically contracted, and this causes a partial or complete obstruction (Hirschsprung disease). The loss of adjacent loci contributes to a wider phenotype (Fewtrell et al., 1994). Deletions in 13q22 and 2q22.3, removing the *EDNRB* and *ZEB2* loci, respectively, are the basis of two other Hirschsprung-associated syndromes (Shanske et al., 2001; Engenheiro et al., 2008); these, and *RET*, more usually present as classic mendelian mutations.

Chromosome 11.

del 11p11.2: Potocki-Shaffer Syndrome.

In 1993, Shaffer et al. described a family segregating an insertional translocation, with 11p11.2 inserted into proximal 13q, and in which some individuals had the 11p deletion after malsegregation of the translocation. The clinical phenotype included the notable features of multiple exostoses, and craniofacial dysostosis with enlarged parietal foramina, along with mental retardation and micropenis in males. This picture resembled that of a patient with acrocephalosyndactyly and an apparently normal karyotype (Lorenz et al., 1990), in whom the same deletion was subsequently demonstrated. Further such cases have since been recognized, typically due to de novo deletion (Bartsch et al., 1996; Potocki and Shaffer, 1996). The parietal foramina are due to haplo-insufficiency of *ALX4*, and the multiple exostoses reflect haplo-insufficiency of *EXT2* (Wu et al., 2000). An exceptional case is described in Chuang et al. (2005) of a del(11)(p11.2) child whose phenotypically normal mother carried the same deletion, but with a supernumerary 11p11.2 neocentromeric marker chromosome, and thus having an overall balanced genotype.

del 11p13: WAGR Syndrome (Wilms Tumor, Aniridia, Genital Defects, Mental Retardation).

Haplo-insufficiency of the *PAX6* morphogenesis gene causes aniridia (absence of the iris). Loss of one *WT1* allele can comprise the first hit in the sequence of events to cause Wilms tumor, and it is also responsible for the impairment of genital development. These two genes, along with some putative brain genes, are removed in the 11p13 deletion, and the tout ensemble adds up to the WAGR syndrome (Fischbach et al., 2005; Xu et al., 2008). 11p13 deletions and translocations with a presumed position effect are the cause of a substantial fraction, about 40%, of all cases of aniridia (Crolla and van Heyningen, 2002). Interestingly, a duplication for the segment 11p12-p13 also produces an eye defect, indicating that a *PAX6* dosage effect, whether an insufficiency or an excess, influences the morphogenesis of the eye (Aalfs et al., 1997).

del 11q23: Jacobsen Syndrome.

This syndrome is of some interest because very rarely the fragile site (FRA11B) at 11q23.3 may predispose to the generation of the deletion (Jones et al., 1994). However, the great majority of patients do not have their deletion breakpoint at or immediately adjacent to the fragile site. Grossfeld et al. (2004) reviewed a large cohort (110 patients), documenting the range of phenotype, and noting the interesting point of a particular type of platelet disorder, associated with neonatal thrombocytopenia, which may be pathognomonic for the syndrome. Van Zutven et al. (2009) review cases due to an interstitial deletion, noting the variability of breakpoints, and including one case of their own due to the mother carrying an insertional translocation 46,XX,ins(4;11)(p14;q24q25).

Chromosome 13.

del 13q14: Retinoblastoma Plus.

The association of retinoblastoma with constitutional 13q– was recognized in the early days of cytogenetics, and this observation gave the clue to the position of the retinoblastoma gene (*RB*) on this chromosome at 13q14. A wider syndrome can accompany the deletion, including mental retardation and facial dysmorphism, the severity according to the extent of the deletion, those extending distally to q14.1 being of more severe effect than those extending proximally (Baud et al., 1999). One 13q– child is recorded with retinoblastoma detected at age 4 and going on to a diagnosis of Wilson disease, an autosomal recessive disorder of copper transport, at age 11. Since the Wilson locus is also in this region (13q14.3-q21.1), the assumption is that the child's other chromosome carried a Wilson mutation, and the deletion “exposed” this mutation in the hemizygous state (Riley et al., 2001). While de novo generation is the rule, five cases are known in which a parent carried an insertional translocation involving 13q14 (Punnett et al., 2003).

del 13q21.1-qter.

A distinct array of malformations is associated with deletions more distally in 13q, and the observations in patients allows karyotype-phenotype mapping of the region (Fig. **19–2**) (Kirchhoff et al., 2009).

Chromosome 14.

del 14q12.

14q12 contains the *FOXG1* locus, and loss of this gene as part of a ~4 Mb microdeletion leads to a syndrome of mental retardation and epilepsy, sometimes called “congenital Rett syndrome”; mutation within *FOXG1* leads to the similar phenotype. The concomitant microduplication is also recognized (Yeung et al., 2009a; Brunetti-Pierri et al., 2011).

Chromosome 15.

del 15q11.2.

This microdeletion, requiring microarray for its demonstration, has the interest that it encompasses the region between the two alternative proximal breakpoints, BP1 and BP2, of the Prader-Willi/Angelman deletion;[11] this particular segment is not subject to imprinting (Bittel et al., 2006). A psychiatric phenotype is frequent. The deletion is often inherited from a less affected parent (von der Lippe et al., 2011).

del 15q11-q13.1: Prader-Willi Syndrome, Angelman Syndrome.

See Chapter 22.

del 15q13.2-q13.3.

It is apparent that “brain genes” reside in 15q13.2-q13.3 (very close to, but nevertheless quite distinct from, the Prader-Willi/Angelman region), which may influence intellect, behavior, and the psyche. Autism and poor behavior are particular observations in the individual with a microdeletion, along with variable mental impairment (which can be severe); dysmorphism, if present, is subtle. Other recognized associations are with epilepsy and with schizophrenia. The deletion typically encompasses the segment between two paralogous breakpoint[11] (BP) sequences: either the 3.95 Mb segment between BP3-BP5, or, more commonly, the 1.5 Mb segment between BP4-BP5 (and see Table 19–2). A number of cases, including both de novo and familial examples, have been identified in the short period of time since the first recognition, and this may be one of the more common of the microdeletion syndromes (Gu and Lupski, 2008; Sharp et al., 2008; Ben-Shachar et al., 2009; Dibbens et al., 2009; Miller et al., 2009; Pagnamenta et al., 2009; van Bon et al., 2009). But not all individuals are affected, and indeed many family members with the same deletion are normal,[12] attesting to an apparent incomplete penetrance of the deletion (Cubells et al., 2011). Thus, this microdeletion may be considered as a predisposing factor, which, in the presence of other as yet undelineated variants, may compromise the integrity of higher functioning of the brain, with varying neurological/psychological/behavioral symptomatology in consequence. A question that gender of the transmitting parent and of the child may be relevant remains open; but thus far, no evidence has emerged for a parent of origin effect; furthermore,

this segment is not known to be subject to imprinting. Homozygosity leads to a considerably more severe phenotype, with an encephalopathy associated with mental retardation, seizures, and movement disorder, and blindness due to retinopathy (Masurel-Paulet et al., 2010).

Table 19–2. Deletion and Duplication Syndromes Having Their Basis within Proximal 15q Rearrangement, Listed According to the Breakpoints That Define the Extents of the Deletion/Duplication (and See Note 11)

WITHIN 15q11.2-q13	**PARENT OF ORIGIN**	
	MATERNAL	**PATERNAL**
Deletion;	Angelman	syndrome
BPs 1–3, 2–3	syndrome	syndrome
Deletion;	Angelman +	Prader-Willi +
BPs 1–4, 1–5		
Duplication;	PDD + autism	Often no effect
BPs 1–3		
WITHIN 15q13.1-q13.3	**NO APPARENT PARENT OF ORIGIN EFFECT**	
Deletion;	Variable MR; ASD; neuropsychiatric	
BPs 3–4, 3–5, 4–5	disorder; minor dysmorphism	
Duplication;	Variable MR; ASD; neuropsychiatric	
BPs 3–5, 4–5	disorder; minor dysmorphism	

ASD, autism spectrum disorder; BPs, pairs of involved breakpoints within this region, numbered 1 through 5; MR, mental retardation; PDD, pervasive developmental disorder.

Angelman + and Prader-Willi + refer to more severe forms of these syndromes

In what may be the first example of a genetically targeted pharmacological management in a deletion syndrome, Cubells et al. (2011) report a substantial improvement in aggressive behavior in an adult male with the use of a drug (galantamine) which acts as an agonist for a specific acetylcholine receptor, the gene for which (*CHRNA7*) is located within the 15q13 deleted segment. It is reasonable to imagine that this drug stimulation enabled maximum activity of the reduced quantum (due to *CHRNA7* haplo-insufficiency) of these receptors on brain neurons, and that the neurons thus stimulated allowed an improvedfunction within the neural substrate that subserves control of behavior.

Chromosome 16.

del 16p11.2.

This chromosomal region is particularly subject to rearrangement, presumably reflecting the existence therein of several duplicons (Bachmann-Gagescu et al., 2010; Barge-Schaapveld et al., 2011). Two major regions subject to deletion in 16p11.2 can be listed: a proximal and a distal segment, the former of ~600 kb, and the latter of ~200 kb. The *~600 kb deletion* presents a clinical picture of autism+epilepsy, although the behavioral phenotype may not meet the formal criteria for autism spectrum disorders (Fernandez et al., 2010; Rosenfeld et al., 2010; Shinawi et al., 2010). Macrocephaly is a frequent observation. There may or may not be a mild degree of dysmorphism. The deletion is more usually de novo, but can be inherited. The more distal *~200 kb deletion* includes the *SH2B1* locus, and this predisposes to severe early-onset obesity (Bochukova et al., 2010). *Larger deletions*, of ~7–9 Mb, extend into 16p12.2, and lead to a phenotype which is associated with dysmorphism and mental retardation with severe language disability (but not autism) (Ballif et al., 2007a; Hempel et al., 2009).

del 16p12.1.

This specific ~520 kb microdeletion, extending from nt 21,854,025 to nt 22,374,785, is another of those that may be nonpenetrant, or less obviously expressed, in a parent, but lead to a phenotype of developmental and language delay and certain physical anomalies, more often craniofacial and skeletal, in the child (Girirajan et al., 2010). The basis of this difference may be that the child has another microdeletion/duplication—a "second hit," or "second-site genomic alteration"—elsewhere in the genome. Some of these second hits may have sufficed, of themselves, to produce a phenotype, but the picture is more severe in the company of the 16p12.1 deletion. Veltman and Brunner (2010) suggest that this could be a more general mechanism, and that some second hits of this type might simply be additive in their effects, while others, by virtue of involving segments that contain genes in the same developmental pathway as those in the first hit, could lead to an exacerbation of the particular phenotypic traits, rather than adding in new traits.

del 16p13.11.

This deletion is another to have been discovered "genotype-first," in five patients from a large cohort screened by microarray analysis (Hannes et al., 2009). It is associated with mental retardation, microcephaly and epilepsy, and in some patients, short stature, cleft lip, and other midline defects. The typical deletion is 1.65 Mb in size and contains at least 15 genes.

del 16p13.3: α-Thalassemia and Mental Retardation.

This is one of two α-thalassemia and mental retardation (ATR) syndromes (the other being an X-linked mendelian condition). In the del(16p) ATR syndrome there is monosomy for a >1 Mb segment, including the α chain globin loci and some brain loci (Gibson et al., 2008). A larger deletion determines a broader phenotype, with tuberous sclerosis and polycystic kidney disease as well as the ATR (Eussen et al., 2000). Smaller deletions, in the range 2.7 to 268 kb, rather surprisingly produce no phenotype other than thalassemia, in spite of the deletion of from 1 to 15 other genes (Horsley et al., 2001). Holinski-Feder et al. (2000) report a notable example of ATR-16 due to a subtelomeric

translocation, which had escaped detection on FISH, and which only came to light after a pedigree analysis showed linkage to 16p (see Fig. **19–9**).

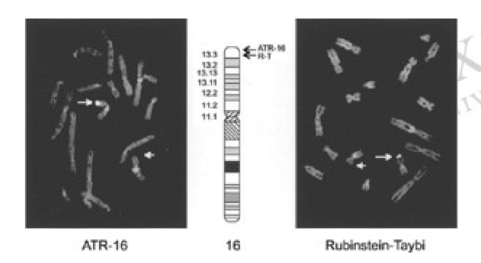


Figure 19–9
Two chromosome 16p deletion syndromes, alpha-thalassemia/retardation syndrome (*left*), and Rubinstein-Taybi syndrome (*right*). Both deletions are in the distalmost band, 16p13.3, the ATR-16 region being distal to R-T. These deletions are difficult or impossible to see on routine banding, but they are clearly apparent on fluorescence in situ hybridization (FISH) with the appropriate probe, as shown here, with one of the no. 16 chromosomes in each case showing nonhybridization (indicated by the shorter arrows).

del 16p13.3: Rubinstein-Taybi Syndrome.

The Rubinstein-Taybi syndrome (RTS) has a distinctive phenotype, and the facies and the broad thumbs are very characteristic. In about one-tenth of cases, the basic defect is a deletion of the gene *CBP* (cyclic AMP-regulated enhancer binding protein) (Wallerstein et al., 1997; Petrij et al., 2000a). It is, thus, a single locus disorder rather than a contiguous gene syndrome, and point mutation within this gene can also cause the syndrome. The basic defect in *CBP* leads to a generalized dysregulation of expression in a number of target genes. The deletion can be seen on FISH using cosmid probes (Blough et al., 2000) (Fig. **19–9**). There is no obvious clinical distinction between those RTS patients with or without the microdeletion (Taine et al., 1998). The range of observed severity presumably reflects a variable expressivity of the abnormal genotype, and the case of identical twins with RTS having rather different neurobehavioral phenotypes supports this suggestion (Preis and Majewski, 1995). The oldest putative case, from 500–900 CE, is that of a skeleton excavated at the Yokem site in Illinois (Wilbur, 2000); some kind of record would be set were this case ever to yield to a paleocytomolecular genetic analysis!

The sites of recombination in the majority of translocation and inversion forms of RTS lie within a breakpoint-cluster region in the 5′ part of the *CBP* gene. This region is also involved in somatic rearrangement, and, for example, the translocation t(8;16)(p11; p13.3) can be a contributory event in the genesis of acute myeloid leukemia (Petrij et al., 2000b). The *CBP* gene thus joins the ranks of the small number of genes known to cause congenital malformation if abnormal during embryogenesis, and cancer if the abnormality is acquired in postnatal life (*NSD1* being another; see section on “del 5q35”).

del 16q24.

Two quite separate syndromes involve band 16q24. First, the *del 16q24.1* syndrome is of interest in that it might cast light upon the basis of some cases of the VACTERL (vertebral, anal, cardiac, tracheo-esophageal, renal, limb) association, given that patient observations included these findings, albeit that the phenotype that led to the discovery of the deletion, namely, the severe lung disorder alveolar capillary dysplasia, is not itself a recognized VACTERL component (Stankiewicz et al., 2009). Second, *deletion of 16q24.3* leads to a syndrome that includes autism, and thus suggesting that the genes implicated, *ANKRD11* and *ZNF778*, might have a role more broadly in autism spectrum disorders (Willemsen et al., 2010).

Chromosome 17.

del 17p13.3: Isolated Lissencephaly Sequence and Miller-Dieker Syndrome.

A deletion of the brain morphogenesis gene *LIS1* produces lissencephaly (“smooth brain”), a severe neuronal migration defect, although agyria/pachygyria (absence/thickness of gyri) may be a more accurate description. Deletions are of variable extent and may be intragenic or remove the entire gene (Cardoso et al., 2002). All FISH-detectable deletions confined to *LIS1* thus far found have been de novo, implying a very low recurrence risk. 17p lissencephaly is to be distinguished from the other major genetic type, the X-linked syndrome (p. 120).

Loss of an adjacent locus or loci, with *YWHAE* an important gene in this respect, adds in defects of other systems and characteristic dysmorphogenesis, and this constitutes the Miller-Dieker syndrome (MDS) karyotype and phenotype (Nagamani et al., 2009) (Fig. **19–10**). The brain malformation is more severe than in the isolated *LIS1* deletion, which may reflect the contribution of another brain morphogenesis locus in distal 17p (Cardoso et al., 2003). Some MDS cases have been due to a parental rearrangement (inversion or translocation), not necessarily recognizable on routine cytogenetic testing, and this possibility should be carefully assessed (Yokoyama et al., 1997; Joyce et al., 2002). 17p13.3 deletions that include *YWHAE* but not *LIS1* present different phenotypes (Nagamani et al., 2009).

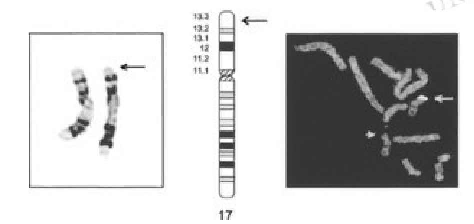


Figure 19–10
Miller-Dieker syndrome. Chromosomes showing deletion in distal 17p, at band p13.3. Fluorescence in situ hybridization (FISH) probe does not hybridize to the deleted chromosome (shorter arrow).

del 17p13.1.

Deletions in this region may be of differing extents, but a point of common interest is loss of the *TP53* gene. This raises a question of susceptibility to Li-Fraumeni syndrome; but, thus far, no such diagnosis has been made (Schluth-Bolard et al., 2010) (and see p. [link]).

del 17p12: Hereditary Pressure-Sensitive Neuropathy.

Hereditary pressure-sensitive neuropathy (HPSN), with alternative names of hereditary neuropathy with liability to pressure palsies (HNPP), and tomaculous neuropathy, is the reciprocal deletion of Charcot-Marie-Tooth neuropathy (see later). The deletion of a particular “nerve gene”—the *PMP22*, or peripheral myelin protein 22 gene—leads to

abnormal myelination of the peripheral nerves, and this compromises their function. A typical presentation is the backpacker who complains of numbness (sensory nerves) and weakness (motor nerves) in the arms after a day's hiking, and these symptoms are due to the pressure of the shoulder straps on the nerves leading to the arms. Almost all HPSN is due to this type of deletion, and thus detectable using FISH, and by microarray (Fig. **19–11**). The deletion can arise de novo, or, as is more usual, can be transmitted from an affected parent. The risk to transmit the defect is 50%. A single family is on record in which the defect was due to a reciprocal translocation, t(16;17)(q12;p11.2), which disrupted the *PMP22* gene, and a heterozygous mother and son had HPSN (Nadal et al., 2000).

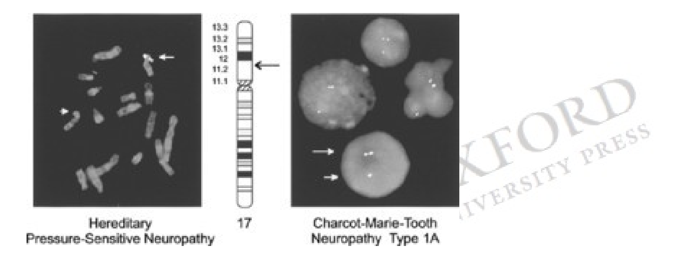


Figure 19–11
Countertype deletion and duplication in 17p11.2 leading to hereditary pressure-sensitive neuropathy (deletion, *left*) and Charcot-Marie-Tooth neuropathy (duplication, *right*). The deletion is evident due to nonhybridization of the appropriate probe on fluorescence in situ hybridization (FISH) of metaphase chromosomes (shorter arrow, left). The duplication can only be discerned when the chromosomes are in their attenuated state in interphase, and two separate (but close) spots identifying the duplicated segment be appreciated (longer arrow, right) in a substantial fraction of cells, while the normal chromosome shows a single spot (shorter arrow).

Two distinct sex-dependent mechanisms are involved that produce the deletion or duplication of the chromosome 17 region associated with, respectively, HPSN and Charcot-Marie-Tooth type IA (Lopes et al., 1998; Inoue et al., 2001a). Rearrangements arising from maternal gametogenesis, which can be either deletions or duplications, are due to an intrachromosomal mechanism, either an unequal sister chromatid exchange or, in the case of deletion, excision of an intrachromatid loop. If the rearrangement occurs in paternal gametogenesis (the more common scenario), it comprises a duplication and arises by unequal meiotic crossing-over between the two no. 17 chromosomes (cf. Fig. **19–1**), an interchromosomal mechanism.

HPSN is an example of the unusual circumstance of the monosomic state having a less severe phenotype than the trisomic: the neuropathy of Charcot-Marie-Tooth disease is more disabling than in HPSN. This might reflect an accumulation within the cell of degraded excess protein in the *PMP22* duplication, compromising the cell's function, versus a mere reduction in the amount of protein with the deletion (Ryan et al., 2002). HPSN is also an example of a deletion that occurs in an otherwise almost gene-bare region. Thus, the phenotype is essentially based upon the dosage effect of just the one gene, *PMP22*.

del 17p11.2: Smith-Magenis Syndrome.

The Smith-Magenis syndrome (SMS) encompasses a picture of dysmorphology, mental defect, and fractious behavior (Fig. **19–12**). Sleep disturbance (associated in most cases with a reversal of the normal circadian pattern of melatonin secretion, but the neurobiology remains to be clarified; Boudreau et al., 2009) is a characteristic feature; and a habit of self-mutilation, and a markedly diminished pain sensitivity, can manifest as “onychotillomania” (pulling out nails) (Smith et al., 1998a; Potocki et al., 2000b). To the practiced eye, the facies may be distinctive (Allanson et al., 1999). Most (>90%) patients have a ~4 Mb deletion, resulting from anunequal meiotic crossover followed by nonallelic homologous recombination, the stage having been set by the existence of flanking low-copy repeats (Fig. **19–1**); a larger deletion may be associated with a more complicated phenotype (Natacci et al., 2000). The crucial locus within the deleted segment is apparently the retinoic acid induced protein 1 (*RAI1*), haplo-insufficiency of which may compromise the activity of a number of downstream genes, and each of these, thus compromised, then contributing to a component of the syndrome (Girirajan et al., 2009). No instance is known of recurrence in a family of SMS due to the common deletion.

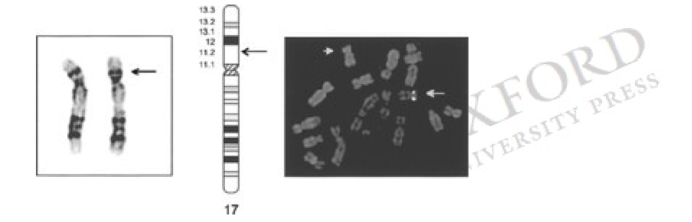


Figure 19–12
Smith-Magenis syndrome. Chromosomes showing deletion in proximal 17p, at band p11.2. Fluorescence in situ hybridization (FISH) probe does not hybridize to the deleted chromosome (shorter arrow). This region is proximal to the segment deleted in hereditary pressure-sensitive palsy (Fig. **19–11**).

del 17q21.31.

This is a condition that could, in retrospect, be seen as a syndrome, but in which the collection of features did not impress sufficiently that recognition was likely to have been achieved ahead of the laboratory discovery (“genotype-first”) of this recurrent deletion. Poor speech development, epilepsy, and a friendly disposition have led some to see a resemblance with Angelman syndrome. There are only four definite genes in the deleted region; and it remains to be learned whether this is a contiguous gene syndrome or essentially a single-locus disorder, the remaining genes being dosage insensitive (Grisart et al., 2009; Tan et al., 2009).

del 17q23.1q23.2.

This is another genotype-first syndrome, in which nonallelic homologous recombination is the usual basis of the microdeletion (Ballif et al., 2010). Heart and limb defects are characteristic, which may be due to haplo-insufficiency of the transcription factor genes *TBX2* and *TBX4*.

Chromosome 18.

del 18p, del 18q.

Quite substantial deletions of chromosome 18 short arm and long arm were recognized in the very early days of medical cytogenetics; the small size of this chromosome facilitated recognition of these deletions on “solid-stain” cytogenetics (De Grouchy et al., 1964; De Grouchy et al., 1966).

Two-thirds of *18p deletion* cases are due to a de novo simple terminal deletion, the remainder reflecting de novo rearrangement, or malsegregation of a (possibly cryptic) parental rearrangement (Wester et al., 2006; Turleau 2008). Familial cases are on record (Maranda et al., 2006). The phenotype has been refined, and attempts made at a

clinical correlation according to the nature of the deletion; sophisticated studies may be necessary to distinguish simple terminal deletions from more complex rearrangements. A very small (subtelomeric) 18p deletion may produce a less severe physical and functional neurological phenotype, but including important psychopathology (Babovic-Vuksanovic et al., 2004).

One particular locus in the *18q deletion* whose haplo-insufficiency may contribute to the mental defect is *MBP* (myelin basic protein) at 18q23. Gay et al. (1997) studied twenty 18q– patients with brain scanning, in order to determine how well the white matter of the brain was developed. In 19 patients in whom the *MBP* gene was included in the deletion, reduced myelination was demonstrated, whereas in the one patient whose deletion did not include *MBP*, a normal appearance of the cerebral white matter indicated that myelination had proceeded, at least grossly, without compromise. Array-CGH has enabled a more precise genotype-phenotype correlation of this dysmyelination, and certain other associated traits (kidney malformation, aural atresia, failure of response to growth hormone, and has also increased our understanding of the variability of the underlying rearrangements (Cody et al., 2009; Heard et al., 2009).

Chromosome 19.

del 19p13.3-pter; del19p13.13; del19p13.12; del 19q13.11.

Chromosome 19 is gene-dense (Color plate Fig. 3–10), and it is not surprising that this chromosome has been little represented in the ranks of deletion syndromes. Nor is it surprising that these microdeletions are small, and require subtelomeric FISH or array-CGH for their recognition and delineation, as Archer et al. (2005), Malan et al. (2009), Schuurs-Hoeijmakers et al. (2009), Dolan et al. (2010), and Bonaglia et al. (2010) illustrate, with respect to the four recorded syndromes, del(19)(p13.13), del(19)(p13.12), del(19)(p13.3-pter), and del(19)(q13.11). Yet further variations upon this theme come from very small deletions, such as the interstitial 174 kilobase deletion in 19p13.3 described in de Smith et al. (2011), which, while very likely causative, it cannot yet definitely be assumed that the associated phenotype is due to the detected imbalance.

Chromosome 20.

del 20p12: Alagille Syndrome.

The characteristic features of this syndrome are stenosis of the peripheral pulmonary arteries, and insufficient development of bile ducts within the liver (thus, “arteriohepatic dysplasia”), along with certain eye and skeletal defects, and a distinctive facies (Krantz et al., 1997). Most patients have a mutation in the gene *JAG1*, but a very few have a cytogenetic defect. In one series of 109 subjects, only three had visible chromosomal abnormalities: two deletions, and one unbalanced 4;20 translocation (Crosnier et al., 1999). The phenotype did not vary between those with whole-gene deletions and those with point mutations, suggesting that haplo-insufficiency is the common mechanism. The first report of preimplantation diagnosis by microarray of an Alagille deletion due to a parental t(2;20)(q21;p12.2) appears in Treff et al. (2011b). A single case report exists of transmission from a mosaic parent (Laufer-Cahana et al., 2002).

A more extensive deletion can encompass the region for Wolff-Parkinson-White syndrome, a disorder of cardiac conduction, the key locus being *BMP2*. The deletion of both *JAG1* and *BMP2* produces a syndrome combining the defects of each (Lalani et al., 2009).

del 20q13.33.

This deletion is yet another that was formerly missed on classical cytogenetics, and subsequently revealed on microarray (Béri-Deixheimer et al., 2007; Kroepfl et al., 2008). In the case of Kroepfl et al. of a dysmorphic and mentally retarded child, the deletion was further delineated on FISH analysis using 20q-derived BAC clones, and then with quantitative RT-PCR interrogating the region of the two breakpoints. Thus, and as we are coming to see more frequently, the description achieves the ultimate degree of precision: a deletion extending from one defined nucleotide position to another, in this case, from nt 62,209,440 to nt 62,301,971. This is a segment of only 92,531 nucleotides, and thus of the two genes contained therein (*MYT1* and *PCMTD2*), one or both can be taken as the causative basis, due to being in the haplo-insufficient state, of the clinical phenotype. Béri-Deixheimer et al. (2007) describe two cases of de novo 20q13.33 deletion. Detailed mapping was performed by microarray CGH in one patient, and confirmed by FISH in both patients. They propose, based on their cases and review of the literature, two clinically distinct phenotypes: one with mild mental retardation, and the other a more complex and severe phenotype.

Chromosome 21.

del 21q: Partial Monosomy 21.

There are 21q deletions of varying degree, and a genotype-phenotype exercise allows an assessment of the contribution of different haplo-insufficient segments to the observed range of phenotypes (Roberson et al., 2011). Deletion of the segment encompassing the *APP* (amyloid precursor protein) and *SOD1* (superoxide dismutase-1) loci is particularly important (Chettouh et al., 1995). Most cases are sporadic, but some have occurred in the setting of a parental balanced translocation (Huret et al., 1995). (Full monosomy 21 has, in the past, been reported; but restudy with more powerful methodology has shown these to be, in fact, partial 21 monosomies due to unbalanced translocations; West and Allen, 1998.)

Chromosome 22.

del 22q11.21-q11.23: 22q11 Deletion Syndrome.

Before their common cytogenetic basis (Fig. **19–13**) was understood, the 22q11 deletion syndromes had a number of labels, including DiGeorge syndrome (DGS), velocardiofacial (VCF) syndrome, and Shprintzen syndrome (De Decker and Lawrenson, 2001). DGS was the name typically applied to a child with heart defect, parathyroid abnormality, and immunodeficiency; in Shprintzen syndrome a cleft or deficient palate was the notable feature; while VCF syndrome emphasized the facial appearance, along with palatal (“velo”) clefting and a heart defect. What used to be called Kousseff syndrome we can now see as 22q11 deletion with the phenotype including neural tube defect (Forrester et al., 2002), and the Cayler syndrome of asymmetric crying facies plus cardiac outflow defect is another variation on the theme (Akcakus et al., 2003). In true acknowledgment of the first definition of the syndrome, in the Czechoslovakian literature in 1955, Sedlácková syndrome may be the most fitting (Turnpenny and Pigott, 2001). With a birth incidence of about 1 in 4000, this is the most common human site of deletion, and this vulnerability likely reflects the nature of low-copy repeats in the 22q11 region. The minimal deletion size is ~1.5 Mb, with some being up to ~3 Mb in extent.

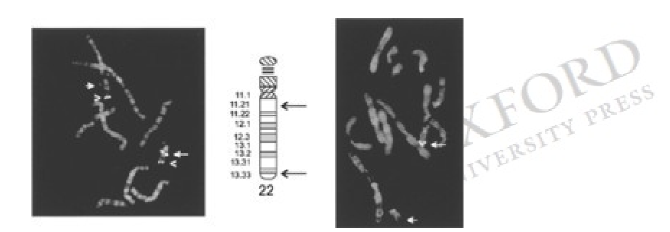


Figure 19–13
Two chromosome 22q deletion syndromes, 22q11 (*left*) and 22q13 (*right*). The DiGeorge critical region probe identifies the proximal 22q11.21 deletion (*left*). One chromosome 22 homolog shows normal hybridization (longer arrow), while the deleted chromosome fails to hybridize (shorter arrow). Arrowheads show control probe. The distal 22q13.33 deletion (*right*) is indicated by the smaller arrow.

Zori et al. (1998) studied a group of patients presenting with velopharyngeal insufficiency, of whom 30% proved to have a deletion, and 13 medical geneticists who studied full-face photographs at a meeting achieved only a 62% correct assignment to del(22q11) or not; repeating the exercise in the 2010s might yield a better result, as the subtleties of the clinical picture become better appreciated. Mehraein et al. (1997) suggest that about a quarter of children with an “isolated” complex cardiac malformation who test positive for a 22q11 deletion in fact show other features of the phenotype (especially the facial/endocrine/immune components), if these are carefully sought. Isolated clefting is not associated with the deletion (and neither with the duplication) (Sivertsen et al., 2007). Psychiatric disorder can be associated, and bipolar disorder and schizophrenia are particular concomitants (Swillen et al., 2000); this may relate to the loss on the deleted chromosome of the microRNA processing gene, *DGCR8* (Stark et al., 2008). Some motor neurological dysfunction (drooling, abnormal speech, dysphagia) may result from a specific failure of development of the motor cortex of the brain (Bingham et al., 1997). In the familial case, a parent can, for example, show mild features of the condition, or have a predominantly Shprintzen facial and palatal phenotype, with a child showing a characteristic DGS cardiac and endocrine phenotype (Devriendt et al., 1997).

There seems not to be a clear correlation between the extent of the deletion and the clinical phenotype, and the fact that most monozygous twins are discordant is a perplexing observation (Amati et al., 1999; Singh et al., 2002b). It may be that there is not one universal DGS gene, but that various combinations of haplo-insufficiencies can lead to a similar, or a not so similar, clinical picture. Attractive candidates as haplo-insufficient genes include *TBX1*, a transcription-factor gene, *YPEL1*, a craniofacial morphogenesis gene, and *GNB1L*, of unknown function (Farlie et al., 2001; Paylor et al., 2006; Prasad et al., 2008; Scambler, 2010).

Most cases are de novo, but about 10% are said to be inherited. It may be that earlier estimates of a larger fraction of affected parents were biased due to studying more remarkable families (Swillen et al., 1998). Indeed, Smith and Robson (1999) report only 5% of parents to have the deletion in an Australian series of 59 cases. Typically, 22q– parents show poor social functioning, and some have frank psychiatric disease. Presumed parental gonadal mosaicism has been described, and prenatal diagnosis using FISH in a subsequent pregnancy will cover this possibility (Sandrin-Garcia et al., 2002). Since the condition is not rare, occasional instances will happen of more than one case in a family, purely coincidentally (Saitta et al., 2004). De novo generation can presumably be invoked in the very rare instance of complementary del/dup mosaicism, as Dempsey et al. (2007) describe in a patient with an atypically small 22q11.2 segment of 1.5 Mb (see also earlier section on “Complementary Deletion/Duplication”).

del 22q11.2.

A rare syndrome due to deletion just beyond the typical 22q11.21-q11.23 site presents a somewhat different clinical phenotype, in one case resembling Goldenhar syndrome; this deletion is due to nonallelic homologous recombination of different LCRs than those involved in the classic syndrome mentioned earlier (Ben-Shachar et al., 2008; Lafay-Cousin et al., 2009). All cases have been de novo.

del 22q13.3: Phelan-McDermid Syndrome

The particular trait is a failure to develop expressive language, and high pain tolerance is also notable; the physical phenotype comprises fairly “soft” dysmorphism. Phelan (2008) provides a detailed description. Most cases may be sporadic, with deletions of size varying from 100 kb to 9 Mb. Inherited translocations and inversions have been identified, and an instance of parental mosaicism. As well as simple deletions, rings (p. 207) and translocations can be the basis (Luciani et al., 2003). Of the contiguous genes involved, loss or compromise of *SHANK3* (also called *proSAP2*) is likely to be an important contributor to the phenotype (Bonaglia et al., 2006). The diagnosis has been made incidentally when a distal 22q internal control probe (ARSA), used at the time of diagnostic testing for the 22q11 deletion, failed to hybridize. Bonaglia et al. discuss the technical difficulty in detecting the deletion, especially when very small, and the advisability of having a low clinical threshold for suspicion, in any child with failure of speech acquisition. An intriguing suggestion is that intranasal insulin may be useful in improving behavior and cognitive capacity (Schmidt et al., 2009).

Chromosome X.

X deletions detectable on classical cytogenetics are discussed in Chapter 13.

del Xp22.3: X-Linked Ichthyosis.

Deletion of the steroid sulfatase (STS) gene at Xp22.3 leads to a pre- and a postnatal phenotype, expressed in placenta and skin, respectively. With a male fetus, the pregnancy is likely to proceed beyond full-term, and there may be the need to induce labor; and in early childhood, the boy develops extensive ichthyosis. A low or absent maternal serum unconjugated estriol (uE3) level in pregnancy is a strong indication of STS deficiency. In one study (Kashork et al., 2002), of nine mothers with low/absent uE3, prenatal diagnosis in six identified (in the male fetus) a complete STS deletion on FISH, and in one, a partial deletion; these seven mothers were all shown to be heterozygous. The reciprocal duplication of the STS locus is a benign population variant in both males and females.

del Xp22.3: X-Linked Kallmann Syndrome.

Failure of normal pubertal development, and an inability to smell, are the hallmarks of Kallman syndrome (KS). The *KAL1* gene at Xp22.3 controls the migration of two classes of neuron to their proper place in the hypothalamus: neurons that would normally produce a hormone (GnRH) to trigger the pituitary to release sex hormones, and neurons in the olfactory pathway. Only a minority of KS is X-linked and due to the *KAL1* locus (other loci are autosomal), and of these, most are point mutations. So only infrequently will a FISH-detectable Xp deletion be found. Deletions of wider extent may include the steroid sulfatase (see earlier, *del Xp22.3*) and ocular albinism loci, producing a wider clinical picture (Hou et al., 1999). Small duplications of *KAL1* are found in the population as a normal variant.

Duplications

While there are certainly very many individual duplication cases on record, rather fewer duplication phenotypes have acquired eponymic (or acronymic) status than with deletions, and we do provide a somewhat shorter catalog than the listing of deletion syndromes earlier. Some have a countertype deletion, listed earlier (and see Table 19–1); in the case of those with no recorded reciprocal deletion, it may be that haplo-insufficiency is nonviable.

Chromosome 1.

dup 1q21.1.

The phenotype of this microduplication includes, as does the deletion for the same segment (see earlier), mental retardation and autism (Mefford et al., 2008). As the microdeletion leads to microcephaly, so does the microduplication cause macrocephaly (Brunetti-Pierri et al., 2008).

Chromosome 2.

dup 2p22p25.

A locus or loci controlling the process of neural tube formation apparently resides in 2p24. Duplication that includes this region may be associated with neural tube defect (NTD), spina bifida or anencephaly, and perhaps the duplication map in Figure 3–11 (in Chapter 3) should be updated in this respect. 2p24 may be the most focused “NTD region” in the karyotype, although a few other putative chromosomal sites of NTD susceptibility are recorded (Thangavelu et al., 2004).

dup 2q37.

The 2q37 duplicated state may be associated with intellect within the normal range, and little or no dysmorphism (Batstone et al., 2003), in contradistinction to the deletion (see earlier).

Chromosome 3.

dup 3q29.

The reciprocal duplication of the 3q29 microdeletion syndrome (see earlier) has the notable feature that the majority of cases are familial, in contrast to the deletions, which arise de novo (Ballif et al., 2008; Lisi et al., 2008). As with the deletion, mild to moderate mental retardation and microcephaly are the most commonly observed traits in index cases. The normality of a transmitting parent, which is sometimes observed, may reflect an incompletely penetrant genotype, and other genetic factors may influence expression, as per the concept discussed in Veltmann and Brunner (2010) earlier with respect to the del 16p12.1.

Chromosome 7.

dup 7q11.23.

This duplication is the reciprocal recombination product to the Williams syndrome (WS) deletion (see earlier section on “del 7q11.23”). Familial cases have been observed. The phenotype is variable, with expressive language delay (in contrast to the loquacity of WS) as a primary component (Orellana et al., 2008; Torniero et al., 2008); this trait was more severely manifest in a child with a triplication of the WS segment (Beunders et al., 2010).

Chromosome 10.

dup 10q24: Split Hand Foot Malformation Syndrome Type 3.

Tandem duplication of the 10q24 region, preferably using array-CGH for its recognition, has been associated in a minority (about 18%) of cases presenting with this malformation syndrome (Everman et al., 2006). One example of sibship recurrence due to maternal somatic-gonadal mosaicism is on record (Filho et al., 2011).

Chromosome 15.

dup 15q11q13.1: Syndrome of Intellectual Impairment and Autism without Dysmorphism.

Duplications of the proximal region of 15q, between breakpoints 1 and 3 (Table 19–2), may determine a syndrome of intellectual impairment, ranging from borderline to severe (“pervasive developmental disorder”), with autism often a prominent feature (Battaglia, 2008; Hogart et al., 2009). Physical findings are usually unremarkable. The frequency may be about 1 in 600 in a referral population with “developmental delay” (Thomas et al., 2003b).

The typical duplication includes, but is not as extensive as, the chromatin present in the large idic(15) (see earlier), and the phenotype apparently reflects this, in being not quite as severe as with the idic(15) (Torrisi et al., 2001). The Prader-Willi/Angelman critical region (PWSCR/ASCR) is contained within the duplicated segment, a point of practical usefulness in laboratory analysis. FISH analysis typically shows two spots on the abnormal chromosome, although in some instances molecular analysis is necessary to prove the point (Thomas et al., 2003a). These dup(15)s have the same breakpoints as in the deletions of the Prader-Willi and Angelman syndromes (PWS, AS), involving misalignment of the same duplicons, and thus representing the countertype of the PWS/AS deletions; both inter- and intrachromosomal mechanisms are implicated, and the process may occur on either parental homolog (Ji et al., 2000; Roberts et al., 2002; Hogart et al., 2009; Molina et al., 2011). (Note that this dup(15) is not to be confused with the 15q11.2-q13 euchromatic variant due to constitutional cytogenetic amplification, which does not involve the PWSCR/ASCR, and which is nonpathogenic; p. 264). A duplication between breakpoints 1 and 6 leads to a more severe phenotype (Kitsiou-Tzeli et al., 2010).

As with the 15q11q13 deletion syndromes (that is, PWS and AS), there is a parent-of-origin effect: the syndrome characteristically results when the duplication is transmitted from a heterozygous mother (or if de novo, it is of maternal generation), but not when it is paternally transmitted (Yardin et al., 2002; Thomas et al., 2003a). A biological correlate of this observation is that the *UBE3A* gene is expressed at a greater level from a maternal-originating duplication (Herzing et al., 2002)[13] A very few cases of abnormal children with a duplication of paternal origin may reflect there having been an atypical imprinting in the region; or the observation may be coincidental.

Given that the phenotype is essentially confined to brain functioning, this is the tissue of particular study relevance. Hogart et al. (2009) measured quantitative reverse transcription-polymerase chain reaction (RT-PCR) transcripts from postmortem material in two dup(15)(q11q13) individuals. In tissue from a male with severe cognitive impairment and seizures, they showed that gene expression and DNA methylation correlated with parental gene dosage. However, in a female with autism and milder PWS-like traits, unexpected deficiencies were seen in paternally expressed transcripts of SNRPN, NDN, HBII85, and HBII52, and the levels of maternally expressed *UBE3A* were unchanged. Their findings suggest that genetic copy number changes, combined with additional (epi)genetic or environmental factors, influence clinical heterogeneity in the 15q11q13 duplication syndromes.

Parental studies are appropriate, in order to enable accurate advice about recurrence, in particular distinguishing de novo from familial, and in the latter, maternal versus paternal origin.

dup 15q13.2q13.3: Syndrome of Neuropsychiatric Disorder and Autism.

As noted in the “Deletion” section, this rearrangement involves a segment very close to, but distinct from, the 15q11-q13.1 segment discussed earlier (Table 19–2). The clinical picture with the duplication is similar to that of the deletion, predominantly one of intellectual impairment, difficult behavior, poor attention, autistic features, and subtle dysmorphism. Counseling is complicated by the incomplete penetrance and variable expressivity of this genomic disorder (Miller et al., 2009; van Bon et al., 2009).

dup 15q26qter: Syndrome of Overgrowth and Intellectual Disability.

A growth control locus, the insulin-like growth factor receptor type 1 (*IGFR1*), is sited within distal 15q, at band q26. Duplication of this locus is associated with a syndrome of which overgrowth is a particular feature, along with macrocephaly and a degree of intellectual compromise (Faivre et al., 2002; Nagai et al., 2002). This contrasts with the growth retardation that characterizes deletions of this region (e.g., the ring 15 syndrome, see p. [link]). From an analysis of four (inherited) cases, Tatton-Brown et al. (2009) delineated the clinical features in some detail, noting a distinctive facial appearance, and that renal anomalies may be frequent.

Chromosome 16.

dup 16p11.2.

This microduplication is characterized by attention-deficit disorder and poor behavior, speech delay, and microcephaly; dysmorphism, if present, is mild. Parental transmission is frequent, and some relatives may be apparently unaffected (Rosenfeld et al., 2010).

dup 16p13.3.

This countertype of the Rubinstein-Taybi syndrome (RTS) deletion leads to a recognizable phenotype, and like RTS, the key gene whose duplication is substantially responsible for the clinical picture may be *CBP* (Marangi et al., 2008; Dallapiccola et al., 2009). In a series of 12 cases in Thienpont et al. (2010), 10 were de novo, and 2 came from an unaffected parent, pointing to an occasional nonpenetrance.

dup 16p13.11.

Albeit that Hannes et al. (2009) concluded that no phenotype resulted from the 1.65 Mb duplication that is the reciprocal of the deletion (see earlier), Williams et al. (2010) observed an association between a somewhat smaller (824 kb) duplication and attention-deficit hyperactivity disorder, and they refer also to an association with schizophrenia. Reports such as these are likely to spur further CNV studies in these and other psychiatric and behavioral disorders, and the counselor will need to keep a weather eye open.

Chromosome 17.

dup 17p13.3.

Duplication of the "Miller-Dieker region" leads to a less severe phenotype than does the deletion (Roos et al., 2009). Although there is (completely unsurprisingly) mental retardation, there is no gross neuronal migration malformation. One child had a normal brain scan at age 4 years; another infant had a possibly increased signal on scanning in the periventricular region (which is the site of origin of the cortical neurons), and a hypoplastic corpus callosum.

dup 17p12: Charcot-Marie-Tooth Neuropathy.

The most common form of Charcot-Marie-Tooth neuropathy (CMT1A) is due to the duplication of about 1.7 Mb in 17p12, which encompasses the *PMP22* (peripheral myelin protein 22) gene (Nelis et al., 1999).[14] It is the countertype of the deletion (see earlier), which causes pressure-sensitive neuropathy. The duplication leads to the production of a 150% amount of the PMP22 protein, and this excess mars the capacity for proper functioning of the peripheral nerve. The major effect is on the motor nerves, and weakness is the important consequence. The nerves to the peroneal muscles (on the outside of the leg, with tendons passing around the ankle to the foot) are particularly vulnerable, and an alternative name for the condition is peroneal muscular atrophy.

The laboratory diagnostic test was initially based upon FISH, which had the benefit of giving a direct visual demonstration of the duplication, with probe hybridizing twice to the duplicated chromosome, and seen as two adjacent fluorescent spots, with the third spot due to fluorescence from the other chromosome appearing elsewhere in the nucleus (Shaffer et al., 1997a). Now, MLPA is used in a number of laboratories; and we expect that, in due course, targeted microarray will become the routine approach. (Other genetic forms of CMT are of course not recognized by these tests.)

An intriguing example is that of a de novo X-autosome translocation 46,X,der(X)t(X;17)(p22.1;p11.2) in a mildly retarded female who had CMT (King et al., 1998). The extra segment of 17p attached to Xp produced an attenuated picture of partial 17p trisomy, presumably reflecting an extension of inactivation into the 17p segment from the X-inactivation center of the der(X). The *PMP22* gene on the 17p segment was apparently fully functioning, however, since the neuropathy was typical for CMT. The inactivation process could be supposed to have "jumped over" the *PMP22* region (this process discussed on p. 117). Another rare circumstance is that of homozygosity for the duplication: effectively, a partial 17p tetrasomy. This leads to a more severe manifestation of the neurological phenotype (Pareyson et al., 2003).

Lebo (1998) proposes that prenatal diagnosis should be made available for CMT, notwithstanding that CMT can be a relatively mild handicap, and comments: "Given the slow rate of progress toward curing all forms of human genetic disease, patients with degenerative diseases who already have irreversible nerve pathology should not be offered undue hope for intervention by gene therapy." Couples will make their own decisions.

dup 17p11.2: Potocki-Lupski Syndrome.

This condition has the interesting history that it was predicted to exist, as the reciprocal recombination product of the Smith-Magenis deletion (see earlier) (Potocki et al., 2000a). The prediction was vindicated. The clinical picture is less severe than in Smith-Magenis syndrome, and it includes such rather nonspecific features as mental retardation, infantile hypotonia, and failure to thrive (Potocki et al., 2007).

dup 17q21.31.

The reciprocal of the deletion for this segment (see earlier) leads to a syndrome of microcephaly with severe cognitive compromise and difficult behavior with autistic features, along with fairly mild facial dysmorphism (Kirchhoff et al., 2007; Grisart et al., 2009). Thus, the duplicated state is not notably less severe than in the deletion, as is otherwise quite often observed. This rearrangement (deletion and duplication both) is associated with a particular inversion polymorphism in the parent that facilitates nonallelic homologous recombination between low-copy repeats flanking the region. Thus far, all cases have been de novo.

Chromosome 22.

dup 22q11.2.

The countertype of the common del(22q11) is a duplication for the same segment (Ensenauer et al., 2003; Portnoï, 2009). Theoretically, the dup(22q11) should be similarly common, but it is not—or to be precise, it is not commonly recognized. The clinical picture is quite diverse and may include, in common with the deletion, velopharyngeal insufficiency; a specific association may be bladder exstrophy (Draaken et al., 2010; Lundin et al., 2010). It may comprise no more than abnormal behavior and indeed may merge into normality; and thus it is advisable that parents of diagnosed children be karyotyped (Yobb et al., 2005). Brunet et al. (2006) looked at a large group of patients displaying clinical features of the 22q11 deletion syndrome, to determine whether any 22q11 duplications might be found. None were; although, naturally enough, they did discover some deletion cases.

dup 22q13.

The countertype of Phelan-McDermid syndrome presents with developmental delay, very limited language acquisition, and mild dysmorphism (Okamoto et al., 2007).

Chromosome X.

dup Xp11.22p11.23.

This duplication, seen in a number of sporadic and familial cases, and for the most part affecting the sexes similarly, presents a picture of mental retardation with a largely nondysmorphic physical appearance, but it does display a characteristic pattern on the electroencephalogram (Froyen et al., 2008; Giorda et al., 2009) (and see p. [link]).

dup Xq22: Pelizaeus-Merzbacher Disease.

This is a disease of the white matter of the brain and presents a severe neurodegenerative clinical picture. The genetic defect resides in the *PLP* (proteolipid protein) locus at Xq22. Proteolipid protein is a major structural component of myelin in the central nervous system. A quite common mutational basis of Pelizaeus-Merzbacher disease is duplication (or occasionally triplication) of about a megabase of DNA at Xq22 (Woodward et al., 1998). This is analogous to Charcot-Marie-Tooth neuropathy type 1A (see earlier): an additional copy of a gene that produces myelin, but in this case the myelin that sheathes of axons of the central nervous system. While maternal mosaicism is known, most mutations arose from the (unaffected) maternal grandfathers of affected boys, and it thus appears that an intrachromosomal event taking place in male gametogenesis is the usual source of the defect; the mechanism of "fork stalling and template switching" (p. 296) may underlie the generation of the re- arrangement (Lee et al., 2007). As with CMT 1A, interphase FISH in Pelizaeus-Merzbacher disease may show adjacent spots on the X, reflecting the tandem nature of the duplication, although molecular methodology may be taking over as the mainstay of diagnosis, including prenatal diagnosis and carrier assessment (Warshawsky et al., 2006).

dup Xq27.1: X-linked Panhypopituitarism.

An important gene in the development of the pituitary gland is *SOX3*, the locus of which resides in Xq27.1. Some families with X-linked panhypopituitarism (the *pan* referring to involvement of all the component parts of the gland) are due to duplications of Xq27.1, with the additional dose of *SOX3* the presumed cause of the abnormal gland development. In some families, the duplication is large enough to be seen on high-resolution cytogenetics, and these larger duplications are associated with the additional observation of mental retardation (Woods et al., 2005).

dup Xq28: Lubs Syndrome.

A microduplication in distal Xq28, in the region including the *MECP2* and *FLNA* genes, leads to a syndrome of severe mental retardation with a recognizable facies (a narrow, “pinched” nose being typical), and which may include the functional neurological complications of mixed hypotonia/spasticity, and bowel and bladder dysfunction (Clayton-Smith et al., 2009). The duplication can be transmitted by unaffected females, in whom skewed X-inactivation may be protective. Recurrent breakpoints are defined (Bartsch et al., 2010).

Interchromosomal Rearrangement with Duplication: 46,rea

The great majority of these rearrangements arise de novo, presumably from illegitimate meiotic recombination between nonhomologs, and a low (<½%) recurrence risk applies. The possibility of occult parental gonadal mosaicism warrants consideration of prenatal diagnosis.

Supernumerary Marker Chromosomes: 47,+mar

Parental mosaicism is unlikely, but not completely excluded, if the parental lymphocyte karyotypes are normal. Although a risk for recurrence will be “small” (which we cannot precisely define, although we presume a low single-digit percentage figure, possibly <1%), prenatal diagnosis may be appropriate.

Isochromosomes (Nonacrocentric)

A couple having had a child with an isochromosome, for a chromosome other than an acrocentric, can generally be given encouraging advice, more especially if the child is mosaic. The major mechanisms of generation are considered to operate either at meiosis II or postzygotically, and in either case no discernibly increased risk would be implied. Exceptions to this (noted in the earlier “Biology” section), presumably due to parental gonadal mosaicism, are extremely rare. The unbalanced “Robertsonian isochromosome” is a different category and is to be seen in a different light (Chapter 7).

Triplication.

With very few cases reported, an empiric recurrence risk cannot usefully be determined for the typical sporadic case. Parental gonadal mosaicism remains a possibility (perhaps even a probability), as illustrated by the example in Eckel et al. (2006) of a phenotypically normal mother with a 46,XX,trp(12)(pter→p11.22::p11.22→p12.3::p12.3→qter)[6]/46,XX[44] karyotype, her son having the triplication in nonmosaic form. Such an example obliges caution. At the microarray level, rare instances are reported of a nonpathogenic duplication in a parent leading to a pathogenic triplication in his or her child (p. 267).

Notes:

[1] This word can be, and is, used in the context of a single locus. It is usually applied in cytogenetics to the more recently delineated “microdeletion” syndromes, but it can in principle refer to the classical syndromes with larger deletions.

[2] For the record, the first such case, from Australia, was reported as “incomplete trisomy” (Ilberry et al., 1961).

[3] But one should be aware that low-level mosaicism for a SMC could be missed by array-CGH (Ballif et al., 2006, 2007b).

[4] Magenis et al. (1999) record the historical point that Pallister-Killian syndrome was first identified serendipitously, when fibroblasts taken for archival purposes were subject to routine cytogenetic analysis.

[5] The band p10 (or q10) is sometimes noted, as for example, in 47,XX,+i(12)(p10), indicating that each arm of the isochromosome comprises the entire p (or q) arm.

[6] The presence of two supernumerary idic(15) chromosomes, or one marker with four copies of the PWSCR/ASCR, offers the rare opportunity to use the word “hexasomy” (Qumsiyeh et al., 2003; Hoppman-Chaney et al., 2010). The same principles apply in terms of presence/absence of *SNRPN*, with the abnormal phenotype being aggravated in the hexasomic state.

[7] Diaphragmatic hernia has also been associated with deletions at 8p23.1 and 15q26.2, and thus these segments should be specifically interrogated when a diagnosis of Fryns syndrome is being considered clinically (Slavotinek et al., 2005).

[8] *NSD1* has a quite different role in cancer, as one of the two genes at the breakpoints of a *somatic* translocation t(5;11)(q35;p15.5) that leads to the production of a chimeric protein that may well be an initiating factor in childhood acute myeloid leukemia (Jaju et al., 2001).

[9] The custom of removing the apostrophes from the names of authors associated with syndromes has led to the occasional misspelling of this condition as “William syndrome.” Similarly, the terminal s of Edwards and of Sotos is sometimes erroneously dropped.

[10] Another deletion syndrome with exostosis, but due to another locus, is Potocki-Shaffer syndrome (see section on “del 11p11.2”).

[11] There are well-defined breakpoints sited on proximal 15q and numbered 1–5 from proximal to distal. Their similarity of sequence, due to low-copy repeats, predisposes to nonallelic homologous recombination between any two of the BPs, with a consequential deletion or duplication of the intervening segment. BPs 1–2 are more proximal, in 15q11.2, and comprise one of the proximal breakpoints responsible for the typical PWS/AS deletion, the distal one being BP3, at 15q13.1. BP 4 is located in 15q13.2, and BP5 in 15q13.3.

[12] It may be that some parental abnormality is concealed due to lack of knowledge of the family background. Ben-Shachar et al. (2009) observed rather many of their index cases had been adopted, and they speculate that “the adoption in many cases may have been related to cognitive, psychiatric, and/or social issues in the biological parents.”

[13] Intriguingly, the subset of autistic children manifesting the trait of “insistence on sameness” show greater maternal sharing of alleles in the 15q11q13 region (Shao et al., 2003).

[14] The duplicated segment of chromosome 17 is some 1.7 Mb in size, but it is “gene-sparse.” The very few other genes contained therein appear to imply no phenotypic consequence due to their being in imbalanced state (as also noted earlier in the deleted state; see section on “del 17p12”).



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Chromosomal Disorders of Sex Development

Chapter: Chromosomal Disorders of Sex Development
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THE IDEA of a female with XY chromosomes and a male with XX chromosomes may seem a contradiction in terms. Yet to those who have studied the mechanisms of sex development, perhaps what is more remarkable is that most of the time there is a clear association between being XX and female, and being XY and male. The XX and XY embryo are built on a fundamentally similar outline plan, and only as development proceeds do certain modifications evolve. If at any point in this sequential process some genetic instruction is faulty, inappropriate, or cannot be acted on, the direction of sexual development may proceed imperfectly. In the extreme, the opposite path is taken. This latter state is the particular subject of this chapter, with more of a focus upon those forms in which classical and molecular cytogenetics comprise the key diagnostic investigations, although we do touch on some mendelian conditions. We provide categories for “girls and women,” and “boys and men,” according to the phenotypes presented, and the sex that the individual is regarded as being, by the individual or by the individual's parents.

Nomenclature

These conditions are subsumed under the general heading of disorders of sex development (DSD) (Hughes, 2008). The different chromosomal categories may be indicated by reference to the sex chromosome constitution (XX or XY) and the nature of the gonad (testis, ovary, ovotestis, or dysgenetic/streak). The former expressions XX male, XY female, and hermaphrodite are now referred to as particular types of DSD. Genital ambiguity is now simply denoted XX DSD or XY DSD, according to karyotype; clearly these are rather broad descriptors, and more precise detail might usefully be added in individual cases. With reference to male or female sex, these different levels of definition can apply: gonadal sex (ovary, testis, ovotestis, streak); anatomical/genital sex (structure of the internal and external genital tract); karyotypic sex (46,XX, 46,XY, or other); and behavioral sex (gender identity).

Biology

Somewhat simplified, the fundamental plan of the reproductive tract is that bilateral gonads, arising from the genital ridge, connect with bilateral paired internal ducts (müllerian and wolffian), which enter a midline genital sinus, opening at the perineum. This opening is buttressed on each side by labioscrotal folds and capped above by a phallus. The basic plan of the genital ridge is laid down according to instruction from, in particular, the *WT1* and *SF1* genes. Thereafter, the direction in which gonadal development proceeds is due to the activity of a number of genes on the sexual differentiation pathway.

The Key Role of the *Sry* Gene

In the *absence* of *SRY*, but with input from *WNT4* and *RSPO1*, the gonad develops into an ovary, and the duct system develops into fallopian tubes and uterus. The genital sinus remains as an opening (the vagina), flanked and surmounted by labia and clitoris. The female state results. If a Y chromosome is *present*—or at least that part of the Y that contains *SRY*, the testis-determining gene—the male direction is taken. The *SRY* gene calls into action, among others, the *SOX9*, *FGF9*, and *MAP3K1* genes, and the gonad becomes a testis. The testis, in turn, secretes hormones, of which androgen influences the genital tract to masculinize, and anti-müllerian hormone causes regression of the female müllerian ducts. A vas deferens forms from the duct system. The phallus enlarges. The labioscrotal folds fuse in the midline and accommodate the descending testes. The male state results.

Disorders of Sex Development in Girls and Women

XY Ovarian Disorder of Sexual Development

The 46,XY karyotype in an otherwise normal girl, with (apparently) completely normal female anatomy, is a very rare observation. A single case is reported of a child who was a compound heterozygote for mutations in the *CBX2* gene, discovered only because of a discordant chromosome finding at prenatal diagnosis (Biason-Lauber et al., 2009). The internal genital tract was normal female, the gonads of normal ovarian appearance, and normal upon histology. It may be that *SRY* requires activation by *CBX2* before it can make its male-determining contribution to sexual differentiation.

XY Disorder of Sexual Development, Complete Pure Gonadal Dysgenesis (Swyer Syndrome)

The rare *familial* form provides a unique example of a mendelian condition that can be inherited in an X-linked recessive, Y-linked, or sex-limited autosomal dominant or recessive mode. In the X-linked forms or autosomal forms, the XY female has a perfectly normal Y chromosome, with a normal *SRY* testis-determining gene; presumably, there is a mutation in a gene (whether this be X-linked or autosomal) controlling a later event in the testicular developmental pathway.

One such autosomal gene has been discovered: the *DHH* gene, of which the locus is at 12q12. Of six apparently nonconsanguineous Mexican-mestizo patients with this form of XX DSD, three were homozygous for a *DHH* mutation, and two of whom, not known to be related, had the same mutation (Canto et al., 2004). (The same gene has been implicated, in heterozygous state, in mixed gonadal dysgenesis; Canto et al., 2005.)

In the Y-linked form, there is a mutation in the *SRY* gene itself. In some Y-hemizygotes, the mutant gene has nevertheless been able to reach a threshold of operation and to induce testis development, while in others with the same mutation it has not. Thus, for example, an XY male with a mutation in *SRY* may be a normal fertile man, while his XY child may be a daughter. The threshold is apparently all or nothing: partial expression, that is to say an intersex state, does not result (Jäger et al., 1992; Imai et al., 1999). A man may be a gonadal mosaic for an *SRY* deletion, as presumably was the father in Barbosa et al. (1995). Two sisters with this form of XY DSD (one with gonadoblastoma) had a deletion of *SRY*, but their father showed a normal *SRY* result; there were three other normal sisters and six normal brothers. Similarly, Schmitt-Ney et al. (1995) describe two XY sisters and their XY half-sister with an *SRY* point mutation, whose father was shown to be mosaic for this mutation.

Sporadic occurrence is usual, and in about 15% of these cases the *SRY* gene has a de novo mutation, or rearrangement, that abolishes its function of testis determination. XY females with an intact *SRY* gene might plausibly have an abnormality of one of the other genes, autosomal or gonosomal, in the testis-determining pathway. Candidates include the genes mentioned earlier (*WT1*, *SF1*, *WNT4*, *RSPO1, SOX9*, *FGF9, MAP3K1*, *CBX2*, and *DHH*) and also *DAX1*, *DMRT1*, *SOX3* (the "ancestor" of *SOX9*), and *TSPYL1* (Hughes, 2008; Vinci et al., 2009). In the fullness of time, we may anticipate that a "DSD chip" will be developed, to test for these several genes; Barbaro et al. (2008) have produced an MLPA kit to interrogate the dosage of a number of relevant genes.

The gonad in this form of XY DSD is dysgenetic, and it is seen as a "streak." The genital tract feminizes. The lack of female sex hormones causes failure of normal pubertal development. Amenorrhea and failure of pubertal development are the usual complaints that lead these girls to seek medical advice. Gonadoblastoma, a premalignant neoplastic change in the dysgenetic gonad, is common, and it may progress to dysgerminoma (Verp and Simpson, 1987; Berg et al., 1989; Lukusa et al., 1991; Uehara et al., 1999a). Familial ovarian malignancy was a notable observation in a sibship of three XY women (the karyotype presumed in two who had died at ages 19 and 20) described in Kempe et al. (2002).

As always, an accurate diagnosis is needed to give useful counseling. It is thus disconcerting that, in a review of 48 women carrying a diagnosis of "XY female," undertaken in a specialist clinic, in only half was the description accurate. In about one-third of women, the diagnosis was inaccurate, and in one-eighth, frankly wrong (Minto et al., 2005). Counselors will want to assure themselves that the information they have about a patient is correct, and they should painstakingly review all the test findings, with appropriate expert advice.

XY Disorder of Sexual Development, Complete Androgen Insensitivity Syndrome

This is a mendelian condition, in which the locus happens to be on the X chromosome. Here, the defect lies further down the developmental path. The gonad becomes a testis and produces testicular secretions, but the genital tract, internal and external, is resistant to the effects of androgen. The inheritance is X-linked recessive, and the locus is the androgen receptor gene at Xq11–12 (Brinkmann, 2001). The individual appears externally very much as a female, but there is amenorrhea and pubic and axillary hair is absent. Internally, the vagina is short, and the uterus and tubes are represented only by remnants; the testes may be in the inguinal canal (Boehmer et al., 2001). Malignancy of the gonad, gonadoblastoma or dysgerminoma, is less of a concern, occurring in a minority, in 9% in one study, although in two series no cancers were found. About 5% may be an average figure; the risk may be more so if the gonad is undescended (Lukusa et al., 1991; Rutgers and Scully, 1991; Collins et al., 1993; Alvarez-Nava et al., 1997; Chen et al., 1999b).

One example is on record in which, in a sense, the X-linkage was directly visible to the cytogeneticist: that is to say, the X chromosome was abnormal, including the region containing the androgen receptor locus. An affected aunt and niece had the karyotype 46,Y,inv(X)(q11.2q27) and the connecting mother 46,X,inv(X)(q11.2q27) (Xu et al., 2003). A unique case is that of androgen insensitivity due to uniparental disomy X in a woman with the XXY karyotype (Uehara et al., 1999b).

Disorders of sex Development in Boys and Men

XX Testicular Disorder of Sexual Development

Most males with 46,XX testicular DSD ("XX males," in former parlance) arise from the presence of Yp material (rarely visible cytogenetically) on one of the X chromosomes (Rigola et al., 2002), from occult XX/XXY mosaicism, or from the inappropriate activity of a gene that is normally switched on only in response to a Y-originating genetic instruction. In about three-quarters of cases the *SRY* gene is present, typically the consequence of an abnormal exchange between the X and Y during meiosis I in gametogenesis in the father, and thus clearly a sporadic event (Weil et al., 1994; Wang et al., 1995). These are referred to as SRY^{+} XX males, or SRY^{+} XX testicular DSD.

The phenotype in SRY^{+} XX testicular DSD is similar to that of Klinefelter syndrome, presumably reflecting the similar basic genotypes of active X^{+} inactive X^{+} *SRY* in the two conditions; however, the male with XX testicular DSD differs in being of normal height and of unimpaired intelligence (Ferguson-Smith et al., 1990). Margarit et al. (1998) describe six SRY^{+} cases due to translocation of Yp material to Xp22.3, in whom different Y breakpoints could be identified, but whose clinical phenotypes were very similar: normal intelligence, normal stature, and testicular atrophy with azoöspermia. In these SRY^{+} males with XX testicular DSD, a more accurate cytogenetic designation would be 46,X, der(X)t(X;Y)—or more fully 46,X,der(X)t(X;Y)(p22.3;p11.2), albeit the exchange is not usually visible on standard cytogenetics—and so there is reference to this entity also in the section on the X;Y translocation (Chapter 6). Rare cases are known of a male with XX testicular DSD in whom the *SRY* gene had been translocated onto a terminal arm of an autosome (Dauwerse et al., 2006; Queralt et al., 2008).

Males with XX testicular DSD and having no *SRY* gene are denoted SRY^{-}. The fact of male development being able to proceed (to some extent, at least), despite the lack of SRY product, presumably reflects an inappropriate activation of the testis-determining cascade in an otherwise normal 46,XX embryo, either as a sporadic stochastic event or due to some genetic predisposition. Concerning the latter, Jarrah et al. (2000) report an extended inbred kindred in which XX individuals with varying degrees of masculinization were present, and they suggest that in this family SRY^{-} XX maleness and XX ovotesticular DSD represented a continuum of the same disorder (and see later section on "Ovotesticular DSD"). Grigorescu-Sido et al. (2005) describe an SRY^{-} XX testicular DSD case with imperfect masculinization, whom they contrasted with the normal maleness of two SRY^{+} XX men. Rare cases may reflect an abnormal dosage of another gene in the sex-determining pathway; *SOX9*, *RSPO1*, and *SOX3* have been implicated in this respect (e.g., Parma et al., 2006).

Three cases are reported of males with 47,XXX chromosomes. In one well-studied example, the man was mildly intellectually disabled, with gynecomastia and hypogenitalism, and severe testicular atrophy on biopsy (Ogata et al., 2001). One X of the three was positive for *SRY*. In addition to an Xp-Yp interchange in paternal gametogenesis that produced the *SRY*-positive X chromosome, a coincidental maternal nondisjunction was responsible for a disomic X ovum. Thus, the combination at fertilization was XX(mat) + der(X)t(X;Y)(pat), giving 47,XX,der(X)t(X;Y) and appearing karyotypically as "47,XXX."

XX testicular DSD has been diagnosed prenatally, following the recognition that the chromosomal and ultrasonographic anatomical genders did not match (Trujillo-Tiebas et al., 2006). (This should however be a carefully considered diagnosis, at least for sporadic cases, as mostly the XX "chromosomal sex" will be due to culture of maternal cells.)

45,X Male

We refer to this rare disorder on p. 134. Most, quite possibly all, "45,X males" have, in fact, a molecular translocation of the *SRY* gene to an autosome or to the X chromosome (and might therefore be thought of as a type of Y;autosome or X;Y translocation). In some, the underlying constitution might actually be an X/XY mosaicism.

Y Isochromosomes

A Y isochromosome, idic(Y)(q11), in mosaic state with a 45,X line, is a rare observation in individuals presenting with a disorder of sex development[1] (Lungeanu et al., 2008). These chromosomes presumably arise in paternal gametogenesis, with loss in an early mitosis or mitoses of the embryo, to produce the 45,X line.

Ovotesticular Disorder of Sexual Development

The term *hermaphroditism*, of classical Greek derivation, is losing some favor among those so diagnosed; and the qualifiers “true” and “pseudo” were always somewhat arcane. These days, we speak of ovotesticular DSD. As that descriptor indicates, the defining feature is that the gonads comprise both ovarian and testicular elements: there may be a testis and an ovary, or one or both may be an ovotestis. The most common karyotype is 46,XX (thus, XX ovotesticular DSD), seen in 60%; one-third have mosaicism with one cell line which includes Y chromosomal sequences, mostly 46,XX/46,XY; a few are 46,XY; and other more rare forms are known (Krob et al., 1994; Queipo et al., 2002). Ovotesticular DSD generally presents as a problem in determining the sex of a newborn infant (Hadjiathanasiou et al., 1994).

Most of the 46,XX cases test negative on peripheral blood analysis for the *SRY* gene, and in some of these the basis of the defect may be sporadic inappropriate activation of the testicular developmental cascade in part of the gonadal tissue during its embryonic formation. Alternatively, an apparent XX karyotype may harbor Y material, as Margarit et al. (2000) show in a woman reared as a boy with hypospadias who went on to have gender change surgery after testing “46,XX.” Several years later, reanalysis revealed a tiny segment of Yp translocated on to the X long arm, 46,X,der(X),t(X;Y)(q28;p11.31). A more common explanation in the 46,XX case may be cryptic mosaicism within the gonad itself, with an island or islands of tissue containing the *SRY* gene (Ortenberg et al., 2002; Queipo et al., 2002). It is a curious and unexplained fact that ovotesticular DSD (mostly with a 46,XX karyotype) is far more common in the South African black population than in Europeans (Wiersma, 2004).

The XX/XY state more usually results due to the fusion of twin XX and XY embryos (XX/XY chimerism). Strain et al. (1998) reported a notable example of iatrogenic ovotesticular DSD, which followed in vitro fertilization, presumably due to an XX and an XY embryo fusing; Malan et al. (2007) reached a similar conclusion in a case diagnosed prenatally, and which could be referred to as “tetragametic chimerism.” Another mechanism is that an ovum might divide symmetrically (instead of budding off a polar body), and the two cells are each fertilized by a sperm (Chen et al., 2005c). A further theoretical route is from the postzygotic loss of the X and of the Y in separate cells of an initially 47,XXY conception (Niu et al., 2002). The basis may be molecular, rather than cytogenetic. For example, one nonmosaic 46,XY case had a postzygotic mutation in *SRY* with SRY^{+}/SRY^{-}gonadal mosaicism (Braun et al., 1993). Presumably the SRY^{+} line was responsible for the testicular elements in the gonad, and the SRY^{-} line for the ovarian elements. A somewhat similar patient is described in Modan-Moses et al. (2003), in whom 46,XX $^{SRY+}$/45,X $^{SRY+}$/45,X $^{SRY-}$mosaicism was associated with a clinical picture of ovotesticular DSD.

Other mosaicisms include XX/XXY and X/XY. Kanaka-Gantenbein et al. (2007) report a boy, regarded as normal except for an undescended left testis, who presented as a 13-year-old with a left scrotal hemorrhage. In fact, the undescended gonad was an ovary, which had actually ovulated, and presumably this had been the cause of the bleed. There was a left hemi-uterus and fallopian tube; the testis, on the right, was dysgenetic. On both blood and testicular biopsy the karyotype was 46,XX/47,XXY; as expected, given the presence of a male gonad, albeit an imperfect one, *SRY* and *AZF* loci were present. In another case, a baby girl presenting with clitoral hypertrophy typed 46,XY on blood, but analysis of the removed dysgenetic gonads revealed X/XY mosaicism (Röpkea et al., 2007). On histological examination, the gonads contained testicular and ovarian elements: the XY state was observed more in the testicular component of the gonad, while cells with only an X predominated in the ovarian fraction.

A single case is recorded of ovotesticular DSD associated with an autosomal cytogenetic abnormality, and this may reflect the effects of an autosomal gene on the cascade of sexual differentiation (Aleck et al., 1999). This child had ambiguous genitalia, with one ovarian and one testicular gonad, and karyotyped 46,XX,rec(22)dup(22q)inv(22)(p13q13.1)mat. Testing for *SRY* was negative. Tomaselli et al. (2008) report the first actual autosomal mutation to be recognized, in the *RSPO1* gene, in a woman with ovotesticular DSD, and concomitant palmoplantar hyperkeratosis. This gene is located at 1p34.3; the mutation was in homozygous state, and her parents were first cousins.

Rare *familial* 46,XX cases of ovotesticular DSD may reflect a mutation, whether autosomal or X-linked, such as *RSPO1* or others in this pathway, that induces the testis developmental cascade to proceed at a post-*SRY* stage. Slaney et al. (1998) describe the case of four 46,XX cousins with abnormal sexual differentiation. Three had 46,XX ovotesticular DSD, and one was a 46,XX male. The putative testis-development gene had been transmitted through two mothers. Affected distant relatives due to a familial X;Y translocation are noted on p. 135.

Sterility is almost universal. But of the 11 pregnancies to women with ovotesticular DSD reviewed in Schultz et al. (2009), extraordinarily, all infants were male. The opposite applied to the 46,XY man with ovotesticular DSD reported in Zayed et al. (2008), who had had surgery for removal of an intra-abdominal testicular seminoma, and which included ovarian elements. At the same operation, a uterus and tubes were identified, and removed. A few years later, he underwent testicular aspiration of the remaining gonad, which yielded sperm: these were used for ICSI, and eventually a pregnancy resulted—and a normal daughter was born.

Rare disorders with extragonadal defects

A number of rare conditions exist in which sex reversal coexists with physical, metabolic, and in some, mental defect. By way of example, one of these is XY female DSD campomelic dysplasia (campomelia refers to long bone bowing) with sex reversal. The usual cause is a mutation within the *SOX9* gene (at 17q24.3q25.1), one of the genes operating on the sexual differentiation pathway and which also influences limb bud mesenchymal development (Wagner et al., 1994). A cytogenetic form of this syndrome is seen in an apparently balanced 17q translocation, which may lead to deletion with haplo-insufficiency, or inactivation, of the *SOX9* locus (see Fig. 19–5 in Chapter 19).

In the other direction, a chromosomal imbalance may lead to male genital development in the setting of an XX gonosomal complement. Thus, Seeherunvong et al. (2004) describe a child with a chromosome 22 duplication, 46,XX,dup(22)(q11.2q13), who was *SRY*-negative and had male external genitalia, with intrascrotal gonads, the only genital abnormality being a first-degree hypospadias. The child also had a number of dysmorphic features and psychomotor retardation. A mendelian condition is XX male DSD with palmoplantar hyperkeratosis, in which homozygosity for *RSPO1* mutation is the basis (the same gene noted earlier with reference to one form of ovotesticular DSD) (Parma et al., 2006).

Microarray methodology can reveal the agency of a sex-determining gene in a DSD individual. A 46,XY girl with a syndrome of ovotesticular DSD, absent uterus, and clitoromegaly, along with skeletal anomalies and developmental delay, had a microdeletion of 3 Mb at 9q33.3, and one of the genes within this segment is *SF1*. Point mutations in this gene (and as mentioned earlier) are otherwise on record in patients with XY DSD. In this girl, haplo-insufficiency of the protein product, steroidogenic factor-1, is the likely primary basis of the gonadal defect. Haplo-insufficiency for another gene in the deleted segment, *LMX1B*, led to the disordered skeletogenesis, and a clinical diagnosis of genitopatellar syndrome was made (Schlaubitz et al., 2007).

Genetic Counseling

XY Disorder of Sexual Development, Complete Gonadal Dysgenesis (Swyer Syndrome)

Familial/Inherited Cases.

XY DSD with gonadal dysgenesis, when familial, is mostly inherited as an X-linked recessive, or autosomal dominant or recessive with expression limited to the XY state. Autosomal recessive inheritance would be improbable in a multigenerational family tree, while on the other hand, this mode would be strongly supported in a single affected sibship with more than one affected, and in the setting of parental consanguinity. In the multigenerational scenario, a clear interpretation of autosomal versus X-linkage may not be possible. The risk to the female carrier (as judged by position in the pedigree) to have an affected child would be a simple 25% if the X chromosome is implicated, but not

readily calculable if a partially penetrant autosomal gene is the cause. Although the XY female phenotype is close to that of a normal female, but of course associated with infertility, some couples may want to consider prenatal diagnosis. The use of cytogenetics (XY) and ultrasound morphology (female external genitalia) would presumably allow detection of the condition. As noted earlier, DSD genes are coming to be identified (e.g., *DHH* in some XY DSD), and genetic counseling will be better underpinned as this knowledge evolves.

The Y-linked form is recognized by the demonstration of an *SRY* mutation carried by the XY girl and her XY father. This circumstance would allow the counselor the rare opportunity to apply principles of Y-linked inheritance with incomplete penetrance to risk estimation. Mutational analysis of the *SRY* gene (including deletion detection) may provide the basis for carrier detection and prenatal or preimplantation diagnosis.

Sporadic Cases.

Advice on the recurrence risk in the sporadic case is less straightforward. If a de novo *SRY* mutation is demonstrated, only paternal testicular mosaicism—which, as noted earlier, has been observed—could imply an increased risk for recurrence. The *DHH* gene as the basis of an autosomal recessive form is mentioned earlier. Again, prenatal diagnosis by chromosomal/ultrasound gender discordance should be feasible.

The rare syndromes of XY female with extragonadal defects need to be judged on their individual merits.

For the XY woman herself, assisted conception is possible if a uterus is present, and a handful of successful pregnancy outcomes, using donated gametes, have been reported (Kan et al., 1997; Dirnfeld et al., 2000; Selvaraj et al., 2002; Ko et al., 2007).

Aspects of Management.

Couples electing not to consider prenatal diagnosis (or to continue a pregnancy in which a positive diagnosis has been made) should know of the importance of two factors in managing these girls. First, the psychosexual orientation of these individuals is female. But with secondary sexual characteristics developing incompletely, and infertility being invariable, their self-image is vulnerable. In discussing the condition with parents, the counselor should note the importance of using language that reinforces their view of themselves as girls and women, and the counselor should avoid using such terms as "genetic male." It may be explained to them, beginning in simple terms in childhood, that a genetic factor prevented their ovaries from developing normally (Goodall, 1991). Pregnancy may be achievable with in vitro fertilization using a donor ovum, as noted earlier. Second, there is a substantial risk of neoplastic change in the dysgenetic gonad. A gonadoblastoma arises in around half of familial XY gonadal dysgenesis. The gonadoblastoma itself is noninvasive, but it is often associated with malignant elements, most commonly dysgerminoma, which do invade. Thus, and given that the gonad does not usefully contribute in terms of hormone production, early (first decade) gonadectomy is advisable (Troche and Hernandez, 1986; Verp and Simpson, 1987; Lukusa et al., 1991).

Considerable publicity in 2009 concerning an athlete who recorded extraordinary times in women's running races at an international meeting put in sharp focus the question of how such people are to be regarded. The unfortunate woman's internal genital state (which apparently included testicular elements) became the subject of public speculation and then of public documentation. The Athletics Federation attempted a resolution, acknowledging her "unfair" but entirely innocent physical advantage as a runner, in allowing her to keep her gold medal, but also awarding a gold medal to the second-placed athlete.

XY Disorder of Sexual Development, Complete Androgen Insensitivity Syndrome

This condition is inherited as an X-linked recessive trait, and the risk of recurrence follows classic mendelian principles. The carrier may be identified and preimplantation/prenatal diagnosis accomplished by molecular analysis of the androgen receptor gene. While complete androgen insensitivity typically has a consistent phenotype within families, allowing for good prediction of the consequences of recurrence, incomplete androgen insensitivity can have variable phenotypes within a family (Boehmer et al., 2001). Issues relating to prenatal diagnosis are discussed in Morel et al. (1994), who also make the interesting but unsurprising point that incomplete forms can imply a worse burden than the complete form, with partially virilized males (known as Reifenstein syndrome) having "considerable psychological distress and poor function in their adult life." Similar considerations with respect to gender orientation in the XY girl, as discussed in the preceding section, apply to complete androgen insensitivity. The risk for neoplastic change in the gonad is less, in the vicinity of 5%, in the case of complete androgen insensitivity syndrome (CAIS). Thus, some propose gonadectomy may reasonably be delayed to allow spontaneous pubertal feminization (Jones, 1978; Verp and Simpson, 1987), although regular clinical and imaging checks would be advisable.

XX Testicular Disorder of Sexual Development

Many XX testicular DSD boys are not diagnosed until after childhood, by which time the parents are likely to have completed their family. Some cases may be recognized at amniocentesis following discordant karyotypic and ultrasonographic sex.

The great majority occur as sporadic events in a family, and in these the likelihood of recurrence is very small. If the child is SRY^+, and the father's X is SRY^-, sporadic occurrence is proven. As for the rare case of the SRY^- XX male, once the postulated gene or genes have been identified, those cases that would carry a high recurrence risk will be able to be identified. If prenatal diagnosis is requested, and the fetus is 46,XX, testing for *SRY* along with an ultrasound assessment of external genital morphology should enable distinction (Ginsberg et al., 1999).

Ovotesticular Disorder of Sexual Development

The considerable majority of ovotesticular DSD represent sporadic cases, these being characterized by a 46,XX karyotype and absence (at least on peripheral blood analysis) of the *SRY* gene, and presumed to reflect an "accidental" activation of the testis-determining cascade during gonadogenesis, or cryptic intragonadal mosaicism, as discussed earlier. In some cases, the cytogenetics (46,XY, 46,XX/46,XY, or other mosaic karyotype) or molecular genetics (*SRY* mutation that is not present in father) may allow a more secure reassurance of nonrecurrence. Recurrence is very rare; but a positive family history would, of course, imply a high risk, and the *RSPO1* gene could be a candidate (see earlier discussion; a dermatological phenotype may aid diagnosis). In the SRY^- form, a handful of families are described in which there is also a sib with XX male syndrome, and these cases may speculatively reflect "leaky mutations" in a gene operating at a point downstream in the cascade of sexual differentiation. A familial X;Y translocation is dealt with on its merits.

If the condition is diagnosed prenatally, and the pregnancy continued, counselors should consult Hughes et al. (2006) for a consensus statement on management of children with DSDs.

Notes:

1 But the usual presentation with the idic(Yq) is infertility in an otherwise normal male (p. 391).







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Chromosome Abnormalities and Genetic Counseling (4 ed.)

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Chromosome Instability Syndromes

Chapter: Chromosome Instability Syndromes
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A DEFECT OF DNA repair is the factor underlying the chromosome instability syndromes, also known as chromosome breakage syndromes (Brewer et al., 1997; Michelson and Weinert, 2000; Taylor, 2001). The “instability” refers to the predisposition of the chromosomes to undergo rearrangement or to display other abnormal cytogenetic behavior. Their inclusion in this book is warranted in that special cytogenetic techniques may have a role in clinical diagnosis and prenatal diagnosis, albeit that direct molecular analysis is having an increasing role, as more is learned of the mutational basis of these syndromes.

The classic chromosome instability syndromes are Fanconi pancytopenia syndrome, Bloom syndrome, and ataxia-telangiectasia. The main cytogenetic features are listed in Table 21–1. They are mendelian conditions, and in each the mode of inheritance is autosomal recessive. There is genetic heterogeneity in Fanconi syndrome, with cells homozygous for one mutation able to correct in vitro cells homozygous for another mutation (“complementation”). We briefly note three other rare mutagen-hypersensitivity syndromes—the Nijmegen breakage syndrome; the immunodeficiency, centromeric instability, facial anomalies (ICF) syndrome; and Seckel syndrome. Proneness to cancer is a common concomitant of several of the breakage syndromes (Duker, 2002). Some of these genes have in common their interaction with the breast cancer susceptibility gene *BRCA1*, their protein products forming a “BRCA1-associated genome surveillance complex” (Futaki and Liu, 2001).

Table 21–1. The Three Classic Chromosome Instability Syndromes

	CYTOGENETIC FEATURES
Fanconi pancytopenia	Increased spontaneous and inducible chromosome breakage
Ataxia-telangiectasia	Increase in chromosome breaks, presence of clones with translocations having specific breakpoints in 7, 14, and X
Bloom syndrome	Increased spontaneous and inducible SCE; increased spontaneous chromatid breakage with production of symmetrical quadriradials

Rare or even unique families with various clinical presentations have been associated with chromosomal instability, and some representatives are mentioned in this chapter. Chromosome instability has been reported as an occasional observation in quite a number of known conditions. This list includes, among others, the Cockayne/cerebro-oculo-facial-skeletal syndrome spectrum, xeroderma pigmentosum, Rothmund-Thomson syndrome, Dubowitz syndrome, and Riyadh chromosome breakage syndrome. But in several the associations are not clear, the relevance for genetic counseling is uncertain (other than in supporting a diagnosis), and we do not consider them here. Likewise, chromosome instability is a feature of many cancers, and it may indeed be a crucial factor in the process of carcinogenesis; but this a somatically acquired attribute, and not of relevance in the present context.

A different cytogenetic observation is that of premature sister chromatid separation. This is a feature of Roberts syndrome, Cornelia de Lange syndrome, variegated aneuploidy syndrome, and Warsaw breakage syndrome, and we make brief mention of these conditions. The genes underlying these disorders code for cohesins, which contribute to the control of sister chromatid segregation at cell division, and thus are dubbed “cohesinopathies.”

Clinical Genetics and Cytogenetics

The three classic chromosomal breakage syndromes, as well as Roberts syndrome, Nijmegen breakage syndrome, and the ICF syndrome, are of autosomal recessive inheritance, and the recurrence risk, for parents who have had one affected child, is 1 in 4. In those rare instances in which parenthood is achievable, the risk to the child will in most cases be very low. Cornelia de Lange syndrome is almost always due to a de novo mutation.

Fanconi Pancytopenia Syndrome

This uncommon disorder of protean manifestation (also known simply as Fanconi anemia [FA]) is the least rare of the breakage syndromes (Tischkowitz and Hodgson, 2003; Kennedy and D’Andrea, 2005). Originally described as a disorder of short stature, characteristic facies, and certain malformations along with progressive bone marrow failure, the picture has now widened. In one-third of FA there are no major congenital malformations, although many of these will have minor anomalies, skin pigmentary abnormalities, microöphthalmia, and growth indices below the 5th centile (Giampietro et al., 1997). Acute myeloid leukemia is a common complication. Some patients whose clinical condition resembles the VACTERL[1] association may, in fact, have FA, and tests for chromosome breakage (see later discussion) can enable the distinction to be made (Faivre et al.,

2005).

Chromosomes show a range of abnormalities, including an increase in chromosome breakage, both spontaneously and upon exposure to DNA cross-linking agents (Fig. **21–1**). There is little or no hypersensitivity to radiation damage. The increase in chromosome breakage after exposure of cells to a cross-linking agent such as diepoxybutane (DEB) provides, when it is observed, a reliable diagnostic test (Esmer et al., 2004; Castella et al., 2011).

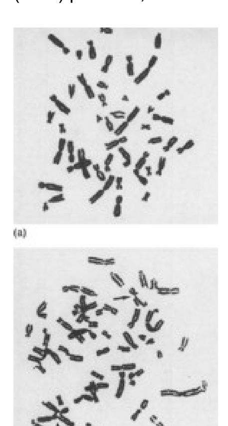






Figure 21–1
Metaphase from (*a*) a control and (*b*) a patient with Fanconi anemia after exposure to diepoxybutane. Note the high level of chromatid breakage in the patient metaphase. One chromatid break is indicated (straight arrow), and a quadriradial figure is shown (curved arrow).

> As Joenje et al. (1998) note, most cytogenetic laboratories will see a case of true FA only very infrequently, and it may be difficult to maintain technical expertise in the practice of clastogen-challenge test protocols. Thus, a negative result might not absolutely exclude the diagnosis. Another reason for a misleading negative result is in vivo "correction" of the functional defect in blood-forming tissue by intragenic homologous recombination, with proliferation of the corrected stem cell population. Joenje et al. refer to patients with typical FA who converted from a positive test result on blood sampling to apparent false-negative over a period of years.[2] Skin fibroblasts maintain the clastogen-sensitive phenotype, and diagnosis following fibroblast study should be reliable.

There is genetic heterogeneity in FA, with at least 12 loci identified, mostly listed sequentially as *FANCA*, *FANCB*, and so on, to *FANCP*. The gene products from these different loci contribute to the control of cellular DNA repair (Kennedy and D'Andrea, 2005; Kim et al., 2011). One of the less common of these genes is the breast cancer susceptibility gene *FANCD1*, better known as *BRCA2*; biallelic mutation leads to a particularly severe form of FA, with a very high cancer risk (Alter et al., 2007).

A risk to the FA heterozygote for cancer has long been suspected (Kennedy and D'Andrea, 2005), and this consideration weighs into the counseling, with respect to the parents and wider families. However, in recent family studies from the United States and the United Kingdom, looking, respectively, at 944 and 575 relatives of patients with FA, there was no suggestion of an increased risk for cancer overall (Berwick et al., 2007; Tischkowitz et al., 2008). The specific genotypes were not assessed in the U.K. study, but the majority were likely to have been due to the *FANCA* genotype, and probably none due to *FANCD1* (*BRCA2*). Three rare genotypes, *FANCC*, *FANCJ*, and *FANCN*, may convey a small increased risk, but confined to cancer of the breast. As for *FANCD1* (*BRCA2*), Alter et al. (2007) observed an inconsistent association with cancer in carriers and concluded that some, but not all FA-associated mutations at this locus convey a cancer risk to the heterozygotes in the family—a conclusion that leads these authors to comment that "counseling those individuals is particularly difficult at present."

Prenatal diagnosis by mutation detection will be possible in those cases with a known mutation. Preimplantation diagnosis has been successfully applied, not only to select an unaffected embryo but also one with the same HLA typing, in order to enable blood stem cell donation to a preexisting affected sibling, an approach not without controversy (Verlinsky et al., 2001b). Otherwise, DEB-induced chromosome breakage in amniotic fluid or chorionic villus cells should provide a satisfactory approach (Auerbach et al., 1986). We have seen a case in which, at routine fetal ultrasonography, upper limb defects were identified, and the couple chose to terminate the pregnancy; subsequent analysis of fetal tissue showed the characteristic cytogenetics of FA. This same cytogenetic testing is being offered in subsequent pregnancies. Merrill et al. (2005) report somewhat similar experiences, although they were able to offer specific testing for a specific mutation enriched in the Jewish population, following ultrasound suspicions of FA.

Bloom Syndrome

Bloom syndrome (BS) is a rare disorder that has its highest prevalence in Ashkenazi Jews, but it occurs in many other ethnic groups. It is characterized clinically by proportionate short stature, a characteristic facies, sun-sensitive skin rash, immunodeficiency, and a marked susceptibility to cancer (German, 1993). Infertility seems to be invariable in the male; females have difficulty conceiving, but a few have given birth (Martin et al., 1994). The Bloom gene, *BLM*, was originally mapped to 15q25-qter by the elegant approach of determining the region of isodisomy in a child with BS and concomitant Prader-Willi syndrome due to uniparental disomy 15 (Woodage et al., 1994). *BLM* codes for a recQ DNA helicase that monitors DNA integrity during S phase (German and Ellis, 2011). (Another member of this gene family is the basis of Rothmund-Thomson syndrome.)

The diagnostic cytogenetic finding in BS is a markedly increased level of spontaneous sister chromatid exchange (SCE). The normal is 6 to 10 exchanges per cell; in BS, it is more than 50 per cell (Fig. **21–2**), although some normal cells may be present in BS patients.[3] The other cytogenetic abnormality is an increased incidence of spontaneous chromatid aberrations, giving the classic symmetrical quadriradial configuration. Intriguingly, this effect can manifest in the haploid state, with the heterozygous male producing an excess of sperm with chromosome breaks and rearrangements (Martin et al., 1994c).

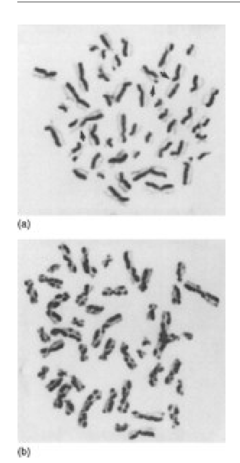


Figure 21–2
Metaphase from (*a*) a control and (*b*) a patient with Bloom syndrome, showing very high sister chromatid exchange (SCE) in the latter. Three points of SCE are indicated (arrows) on the control metaphase.

Prenatal diagnosis may be based upon observation of increased sister chromatid exchanges in chorionic villus cells (Howell and Davies, 1994). Specific mutation analysis would be applicable if the family mutations were known; a Bloom mutation register is maintained (German et al., 2007). For the affected woman's reproductive outlook (in those few surviving to adulthood), the standard mendelian advice, with consideration of the likelihood of the spouse being heterozygous, applies (Chisholm et al., 2001). As noted earlier, the male is infertile.

Ataxia-Telangiectasia

Ataxia-telangiectasia (AT) is the archetype of a group in which the basic pathogenetic process is a failure in one of the monitoring and repair systems that keep watch for DNA damage. The group includes AT itself, and Nijmegen breakage syndrome (below), and both exhibit chromosome instability. The genes for AT and Nijmegen breakage syndrome encode proteins that are part of a complex sensing abnormal DNA structures and monitoring postreplication DNA repair (Michelson and Weinert, 2000).

The clinical presentation of AT is as a brain/immune/cancer syndrome. It is characterized by cerebellar ataxia and oculomotor apraxia (difficulty in performing voluntary eye movements), oculocutaneous telangiectasia, immunodeficiency, and increased cancer predisposition. The cytogenetic hallmarks of AT include frequent nonrandom rearrangements of chromosomes 7, 14, and occasionally X, in T-lymphocytes; nonspecific chromosome breakage in fibroblasts; and normal chromosomes in bone marrow. The breakpoints in the lymphocyte rearrangements are at 7p14, 7q35, 14q12, and 14q32, involving the T-cell receptor and immunoglobulin heavy chain genes. Clones with rearrangements may be harbingers of a T-cell malignancy, and these clones evolve as the disease progresses. Breakage is exacerbated, in vitro, by exposure of cells to ionizing radiation and radiomimetic chemicals such as bleomycin (Kojis et al., 1991).

Most *ATM* mutations are null, but missense and splicing mutations that allow a limited amount of functional product to be produced may lead to milder clinical and cytogenetic phenotypes. Some of these “milder” mutations may, on the other hand, promote an increased cancer risk, including breast cancer in the female heterozygote (Chenevix-Trench et al., 2002).

Prenatal diagnosis of classic AT can be approached cytogenetically on amniocyte analysis, or it may be achievable using direct mutation analysis of the *ATM* gene on chorionic villus tissue (Chessa et al., 1999). Preimplantation genetic diagnosis may be successful (Hellani et al., 2002).

Nijmegen Breakage Syndrome

This is another brain/immune/cancer syndrome, and it is rare indeed. The clinical picture includes microcephaly with brain dysgenesis, immune deficiency, and risk for lymphoreticular malignancy. It shares with AT certain cytogenetic features (preferential involvement of chromosomes 7 and 14 in rearrangements) and radiation hypersensitivity (Antoccia et al., 2006). The causative gene, called *NBS1*, interacts with the *ATM* gene, noted earlier. A founder mutation, 657del5, is common amongst the Slavic population, and most patients are 657del5 homozygotes (Seemanová et al., 2006). Prenatal diagnosis is preferably achieved by specific mutational analysis.

Roberts Syndrome

Roberts syndrome (RS) is a syndrome of craniofacial abnormalities and limb defects that are often severe, and the archetype of the “cohesinopathies”. The phenotype is so very distinctive that is unsurprising that case reports go back some centuries, the first appearing in 1672 (a “Portrait d'un enfant monstre”), well before Roberts' description from 1919 (Bates, 2003; Kompanje, 2009). Intellect is normal. Most affected individuals (about 80%) exhibit a chromosomal phenomenon known as premature chromatid separation (PCS), sometimes described as “tram-tracking” or “railroad track appearance,” and also referred to as “heterochromatin repulsion,” as the sister chromatids bulge away from each other. The gene is *ESCO2* (Vega et al., 2010), and its product enables proper disposition of the chromatids. In its absence, there is an abnormality of sister chromatid apposition around the centromeres, particularly noticeable for those chromosomes with large blocks of heterochromatin (Fig. **21–3**). It is best seen in plain stained or C-banded chromosomes; G-banding obscures the phenomenon (Van Den Berg and Francke, 1993). In this particular instance, classical cytogenetics is the more powerful diagnostic tool, and it may enable recognition of an atypical case; microarray would miss the abnormality (Gerkes et al., 2010).

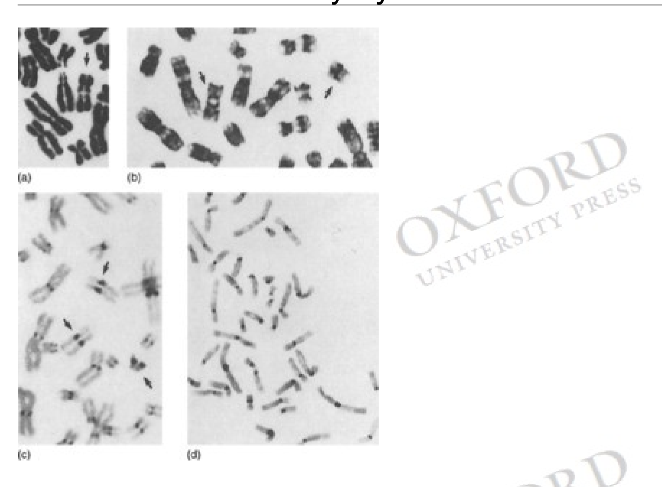




Figure 21–3
Unusual appearance of the chromosomes in Roberts syndrome: puffing at the centromeres (*a* and *b*); a C-banded preparation showing separation of the heterochromatic segments (*c*) is compared with a C-banded preparation from a control showing the normal centromere appearance (*d*). (From N. P. Mann et al., 1982, Roberts syndrome: clinical and cytogenetic aspects, *Journal of Medical Genetics* 19:116–119. Reproduced with the permission of the British Medical Association.)

Prenatal diagnosis based upon the presence or absence of PCS at chorionic villus sampling or amniocentesis, and abnormality or normality of limbs on first-trimester fetal ultrasonography, should be valid in at least the majority. It would be prudent to follow up an interpretation of normality at second-trimester ultrasonography. Molecular testing can be applied when the specific mutation is known (Schulz et al., 2008).

Cornelia de Lange Syndrome

The clinical phenotype of CdeLS is, in the classic case, very distinctive. Three causative genes are known: *NIPBL* (the most frequently seen), *SMC1A*, and *SMC3*. In contrast to the recessive basis of the foregoing conditions, the inheritance with *NIPBL* and *SMC1A* is typically de novo heterozygous mutation, although rare familial transmission is recorded (Russell et al., 2001; Krantz et al., 2004). *SMC3* is X-linked (Musio et al., 2006). Other genes may exist. The cytogenetic phenotype is premature chromatid separation (PCS), and this condition is another cohesinopathy. Testing for PCS may be a useful adjunctive test in the diagnosis of CdLS, if mutation analysis is not available, or is negative (Kaur et al., 2005).

Very Rare Syndromes

ICF (Immunodeficiency, Centromeric Instability, Facial Anomalies) Syndrome.

The ICF syndrome is characterized by immunodeficiency, an unusual facies, and growth and developmental retardation; and a most remarkable tendency of chromosomes nos. 1, 9, and 16 to form "windmill" multiradials by interchange within heterochromatic regions (Fig. **21–4**). This instability of the pericentromeric heterochromatin reflects hypomethylation of satellites II and III, which are important components of its structure. Hagleitner et al. (2008) document the variability of the phenotypic range. The phenotype, physical and cytogenetic, can be considered to be secondary to a failure of methylation. Most cases are due to mutation in the DNA methyltransferase 3B gene, although locus heterogeneity is presumed to exist.

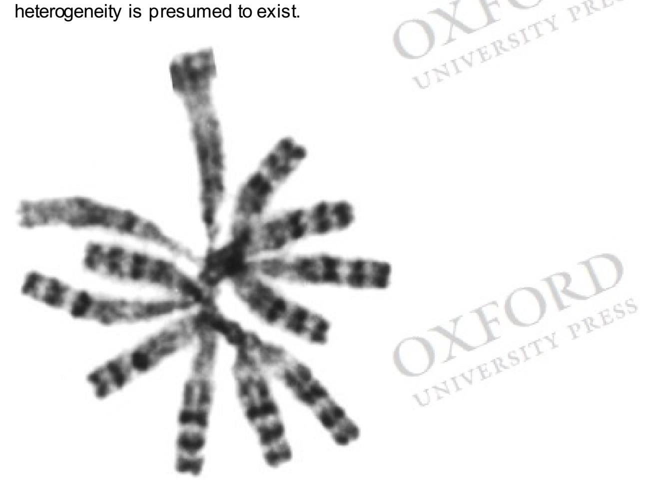




Figure 21–4
A "windmill" or "starburst" multiradial chromosome 1 in the ICF syndrome. (From J. R. Sawyer et al., 1995, Chromosome instability in ICF syndrome: formation of micronuclei from multibranched chromosomes 1 demonstrated by fluorescence in situ hybridization, *American Journal of Medical Genetics* 56:203–209. Courtesy J. R. Sawyer; reproduced with the permission of Wiley-Liss.)

Variegated Aneuploidy with Premature Centromere Separation.

The core phenotype of this recessively inherited syndrome comprises microcephaly with functional neurological abnormality, growth retardation, and susceptibility to childhood malignancy, with most of the lymphocytes and about half of skin fibroblasts showing premature chromatid separation. Many cells are aneuploid, with trisomies, double trisomies, and monosomies, with almost every chromosome represented, and sometimes referred to as "mosaic variegated aneuploidy" (Bohers et al., 2008; García-Castillo et al., 2008). The underlying defect in the cell cycle involves one of the checkpoint proteins (BUB1B) that control progression through the mitotic process, maintaining an alert for chromosome malsegregation. The *BUB1B* heterozygote may display the tendency in a proportion of lymphocytes, and some mitotic cells may present the striking observation of a 92-chromosome count. Prenatal diagnosis has been reported, based on conventional cytogenetics, the abnormalities being very obvious (Plaja et al., 2003; Chen et al., 2004c).

Seckel Syndrome.

At least three loci may exist, *SCKL1–3*. In a group of five patients with this syndrome of growth retardation, microcephaly and characteristic facies, Bobabilla-Morales et al. (2003) demonstrated excessive chromosomal breakage, although not an excess of sister chromatid exchanges. Casper et al. (2004) discovered, in patients with SCKL1 (due to the *ATR* gene, which interacts with *ATM*), that the breaks cluster at common fragile sites. Some SCKL forms do not display chromosome instability.

Syndromes Reported in Only One Family (A Few Examples).

- Ishikawa et al. (2000) reported a single family with a dominantly inherited chromosome instability syndrome. The major clinical observations are mild to moderate mental retardation, depression, and a spastic ataxia, with striking abnormalities of cerebral white matter and the basal ganglia, and an atrophic spinal cord. All three affected individuals having a cytogenetic analysis showed a low frequency of a t(7;14), with a common 14q11.2 breakpoint in each, and a hypersensitivity to radiation and radiomimetic drugs.
- A unique Austrian family appears to present a sex-limited chromosome breakage syndrome with ovarian failure (Duba et al., 1997). The index case had presented with primary hypogonadism, and karyotyping showed a high proportion of cells with breaks, acentric fragments, triradial rearrangements, and dicentric chromosomes. Two healthy brothers had essentially the same chromosome findings. The cytogenetic picture most closely resembled that of Fanconi anemia, and the three siblings also demonstrated an elevation in α-fetoprotein, which is a feature of AT. Lespinasse et al. (2005) report a similar case, but in this instance, a sister and a brother were both infertile, and the α-fetoprotein normal.
- Bakhshi et al. (2006) describe a 17-year-old boy with growth retardation and dysmorphic facies, with mitomycin-sensitive chromosomal breakage, who developed a B-cell lymphoma; they proposed this as a new syndrome, distinct from FA.
- A severely growth-retarded and microcephalic teenager showed both chromosomal breakage and premature chromatid separation, and represents a further cohesinopathy, named Warsaw breakage syndrome for the city of his residence (van der Lelij et al., 2010). The causative gene is *DDX11*, having some sequence similarity to the gene for Fanconi Anemia type J, and coding for a helicase. Inheritance is autosomal recessive, although there is a hint the heterozygote may have an increased cancer risk (and see *FANCJ* mentioned earlier).
- While not a breakage syndrome, the novel disorder reported by Neitzel et al. (2002) deserves a mention. The cytogenetic observation is that of chromosomes prematurely entering mitosis: in metaphase lymphocytes (without colcemid exposure) and fibroblasts, an excess of cells showed chromosomes appearing to be in prophase. The two affected children were severely retarded in growth and mental development. The condition is presumed autosomal recessive.

Notes:

[1] Vertebral, anal, cardiac, tracheo-esophageal, renal, limb.

[2] This reversion to a normal cell line may work as a natural “self-treatment,” whereby the normal marrow clone arising could have a proliferative advantage and ameliorate the disease state (Gross et al., 2002).

[3] Interestingly, the normal cells may be due to a “correcting” genetic event occurring in a bone marrow cell, and which then leads to a heterozygous cell line having a normal in vitro phenotype. The correcting event may be either a somatic recombination between the two sites of *BLM* mutation in the homologs in the BS individual with compound heterozygosity, or a back mutation in a homozygote (Ellis et al., 2001).







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Chromosome Abnormalities and Genetic Counseling (4 ed.)

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Uniparental Disomy and Disorders of Imprinting

Chapter: Uniparental Disomy and Disorders of Imprinting
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UNIPARENTAL DISOMY IS A FASCINATING and important pathogenetic mechanism, albeit that it is the basis of only a small number of well-defined clinical conditions. At the outset, we may list these seven major syndromes:

- Prader-Willi syndrome
- Angelman syndrome
- Beckwith-Wiedemann syndrome
- Silver-Russell syndrome
- Transient neonatal diabetes
- Maternal uniparental disomy 14 (Temple syndrome)
- Paternal uniparental disomy 14

Prader-Willi syndrome, Angelman syndrome, and Beckwith-Wiedemann syndrome can be due to other genetic causes in addition to uniparental disomy (UPD[1]), and for convenience we include a discussion of these other causes in this chapter. As well as the aforementioned seven conditions, certain other UPDs can be the cause of abnormality. These may manifest, in various combinations, the following traits: intrauterine and postnatal growth retardation, intellectual deficit, congenital malformations, and dysmorphic features. In the small print is first, pseudohypoparathyroidism type 1B, due to upd(20)pat, and second (although this may come to demand a larger-print awareness), the maternal hypomethylation syndrome, which has a particular association with in vitro fertilization (IVF) conceptions (Amor and Halliday, 2008). In a category by itself, UPD can be the cause of homozygosity for an autosomal recessive gene. The foregoing notwithstanding, however, the fact remains that most UPDs appear to be without any phenotypic consequence, and a number of syndromes that had seemed fair candidates turned out not to be due to UPD (Kotzot, 2002).

A distinction is to be made between UPD where both chromosomes are identical (uniparental *iso*-disomy, UPID) and where they are different (uniparental *hetero*disomy, UPHD) (Fig. **22–1**). UPD is normally demonstrable only at the molecular level: typically, although not invariably, the UPD pair of chromosomes are cytogenetically normal, and the karyotype appears normal, 46,XX or 46,XY. The pattern of polymorphic DNA markers shows that both chromosomes have the same haplotype as just one of the chromosomes from one of the parents (isodisomy); or the two chromosomes have the same haplotypes as the chromosome pair from one of the parents (heterodisomy). For example, the chromosome 1 haplotypes from parents and child set out in Figure **22–1b** show that the child has two identical copies of one of the father's chromosomes: thus, paternal uniparental isodisomy. This UPD had been discovered fortuitously, when the child was investigated for a clinical diagnosis of congenital insensitivity to pain, an autosomal recessive disorder (Miura et al., 2000). He proved to be homozygous for a mutation in the appropriate gene (*TRKA*, located at 1q21-q22), and his father carried the mutation, but his mother did not. This scenario—a child with a recessive disorder for which only one parent is heterozygous—is commonly the circumstance behind the discovery of UPIDs that would otherwise have been without clinical effect. The other typical route to recognition of harmless UPDs is an incidental discovery in the course of polymorphic DNA marker analysis being done for other reasons.

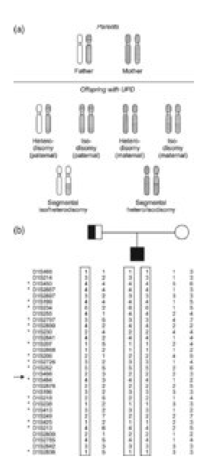






Figure 22–1
(*a*) The distinction between uniparental heterodisomy and uniparental isodisomy. The four parental homologs are shown in different patterns. In the child with *hetero*disomy, the two homologs are different. In *iso*disomy, they are identical. Meiotic crossing-over can lead to segmental iso/heterodisomy, and the pattern can reveal whether the initial nondisjunction had been at meiosis I or II (see text). (*b*) The molecular picture of a child with paternal uniparental isodisomy 1. The markers run from D1S468 at the top of chromosome 1 down to D1S2836 at the bottom. Both the child's chromosome 1 haplotypes are the same, and the same as one of his father's no. 1 chromosomes. He has no chromosome 1 from his mother. (The arrow points to the position of the *TRKA* locus. Homozygosity for an abnormal *TRKA* allele was the cause of his having the recessive condition congenital insensitivity to pain, which had led to his ascertainment.) (From Y. Miura et al., 2000, Complete paternal uniparental isodisomy for chromosome 1 revealed by mutation analyses of the *TRKA (NTRK1)* gene encoding a receptor tyrosine kinase for nerve growth factor in a patient with congenital insensitivity to pain with anhidrosis, *Human Genetics* 107:205–209. Courtesy Y. Indo, and with the permission of Springer-Verlag.)

The state of iso- or heterodisomy can allow an inference as to the site of the initial chromosomal error. Isodisomy typically reflects a meiosis II nondisjunction or a mitotic error, whereas heterodisomy is due to nondisjunction at meiosis I. Partial heterodisomy and partial isodisomy can coexist for the same chromosome pair. For example, a crossover at meiosis I in, say, the distal long arm, followed by meiosis I nondisjunction, could lead to a disomic gamete isodisomic for distal long arm, and heterodisomic for proximal long arm (Fig. **22–1a**, lower right). If the nondisjunction were at meiosis II, the isodisomy and heterodisomy would be the other way around, involving the proximal and distal segments, respectively (Fig. **22–1a**, lower left).

Epigenetics and Imprinting

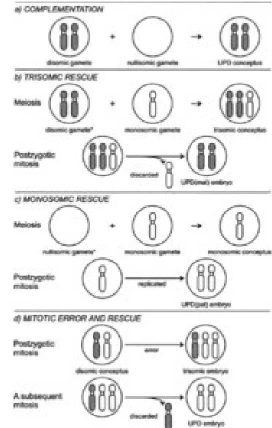
The definition of *epigenetic* has been evolving (Berger et al., 2009). A core consideration is that a phenotype may differ according to whether a DNA sequence is active, or inactive, but the DNA sequence itself remains unchanged. Our focus is on the activity or nonactivity of a gene (or chromosomal segment) according to the parental origin of the chromosome upon which the gene (or segment) is located. Thus, a chromosomal segment can receive an “epigenetic mark”—or is “imprinted”—as it is transmitted from parent to child, depending upon whether it is the mother or the father who has contributed that chromosomal segment, and this determines whether this segment will be genetically active or not active (“silent”). This is spoken of as a “parent of origin” effect. The major physical basis of this epigenetic effect is due to methylation of the DNA (that is, a methyl group attached to cytosine bases), modification of the histone scaffolding of chromatin, and the actions of noncoding RNAs, which severally or separately can then prevent the expression pattern of the relevant gene(s). There are certain chromosome segments (in sum, only a small fraction of the whole genome) that are subject to imprinting.[2] A little counterintuitively, imprinting refers to nonactivity: an imprinted chromosome segment is silenced, while the nonimprinted chromosome segment is the active one.

In the normal setting, with biparental inheritance, imprintable segments (or loci) function monoallelically. That is to say, it is only the segment of maternal origin, or only the segment of paternal origin, as the case may be, which is genetically active. But if both segments originate from one parent, there will be either double the amount (biallelic) of expression, or no (nulliallelic) expression, according to the gender of the contributing parent. (Some imprinting is tissue specific, in which case, the aberrant expression is confined to that tissue.) It is this functional imbalance that is the root cause of the phenotypic effect in the UPD syndromes. If a chromosome is not subject to imprinting, UPD does not of itself cause abnormality, other things being equal. The only other factor due to UPD, and specifically UPiD, which can lead to defect, is homozygosity for a recessive mutation (“isozygosity”), as noted earlier.

Uniparental Disomy for a Complete Chromosome

In UPD for a complete and intact chromosome, both members of a homologous pair come from the one parent. Four routes to lead to this state are the following (and see Fig. **22–2**):

- Gametic complementation
- Trisomic rescue
- Monosomic rescue
- Mitotic error



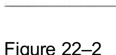


Figure 22–2
Mechanisms whereby complete uniparental disomy (UPD) may be generated. (*a*) Gametic complementation, with one parent producing a disomic gamete, and the other a nullisomic gamete. (*b*) Meiotic nondisjunction in one parent to produce a disomic gamete, with a trisomic conceptus following fertilization, and subsequent mitotic loss of the homolog from the other parent. This is uniparental heterodisomy, from the parent in whom the nondisjunction had taken place. (*c*) Meiotic nondisjunction in one parent to produce a nullisomic gamete, with monosomic conceptus following fertilization, and subsequent mitotic reduplication of the homolog from the other parent. This is uniparental isodisomy, from the parent who had contributed the normal gamete. The reduplication may produce a free homolog or an isochromosome. (*d*) Two sequential mitotic errors.

*Since most meiotic nondisjunction occurs in maternal gametogenesis, these asterisked gametes can be imagined to be oöcytes, with UPD(mat) and UPD(pat) resulting accordingly.

Gametic complementation is mentioned first, as the simplest and classic example, but in truth it must hardly ever be that UPD is the consequence of a meiotic error happening coincidentally in both parents (Park et al., 1998; Shaffer et al., 1998).

Trisomy “rescue” or “correction”[3] is the mechanism behind most UPD. The cause of the trisomy is a typical meiotic nondisjunction that happened in one of the two conceiving

gametes. The rescue process takes place in a cell of the trisomic conceptus at a very early postzygotic stage (possibly even in the zygote), with one of the trisomic chromosomes being discarded, perhaps due to anaphase lag. This enables a cell line within the conceptus to be restored to disomy, but in order to cause the subsequent UPD it is the “wrong” chromosome that is eliminated—that is, purely by chance, the discarded chromosome happens to be the one that came from the normal gamete, and so the remaining two are from the same parent. These two chromosomes will comprise one of each of the homologs of that parent: thus, uniparental heterodisomy. This would be expected to happen, by chance, in one-third of rescues, biparental inheritance being maintained in the other two-thirds (close to these ratios was observed in a large study of UPD16; Yong et al., 2002). The 46-chromosome cell with UPD that results from this process may be the progenitor of the cells which produce the inner cell mass, which in turn gives rise to the embryo. Any remaining trisomic cells may go on to form the placenta, leading to confined placental mosaicism, or they may also contribute to the inner cell mass, leading to trisomy/disomy mosaicism of the embryo. Thus, the phenotypes in some UPD states are complicated by the additional effects of compromised placental function due to trisomy, and/or of fetal trisomy mosaicism.

Monosomic rescue also comes into play following a nondisjunctional event. If a nullisomic gamete is generated at meiosis, then the conceptus will be monosomic (assuming a normal gamete from the other parent). Mitotic correction then takes place, and this is achieved by replication of the single, normal, homolog received from the other parent. In this case, the UPD will be an isodisomy.

The fourth possibility is a *mitotic error* in an initially normal conception, leading to either trisomy or monosomy. In the case of a trisomy, this is followed shortly thereafter by loss, in this cell line, of the nonreplicated trisomic chromosome. In the case of a mitotic nondisjunction resulting in monosomy, the remaining homolog is then duplicated. In both cases, the UPD is isodisomic.

Note that each of these four scenarios requires there to be two separate abnormal events, occurring either simultaneously (the first scenario) or sequentially (the latter three). These errors can be both meiotic (the first), meiotic followed by mitotic (second and third), or both mitotic (the fourth). In whichever case, the original abnormality will practically always have been a sporadic event, with no discernible increased risk of recurrence due to having had one affected child; and indeed, to our awareness, as yet not one instance is known of a recurrence of UPD in the setting of normal parental karyotypes.

One risk factor is known, and this is increasing maternal age. The link here is that meiotic nondisjunction, the root cause of most UPD, is more prevalent in women of older childbearing age. The meiotic errors noted earlier as leading to trisomic rescue and monosomic rescue are typically of maternal origin. Ginsburg et al. (2000) have shown that maternal age is higher in the subset of patients with Prader-Willi, Angelman, and Russell-Silver syndromes due to UPD, compared to those due to other causes. A reduced level of recombination is seen in UPD 15 (Robinson et al., 1998), an observation also made in the classic disorder with a maternal age association, namely Down syndrome. It is worth noting that paternal UPD also has a maternal age effect, which seeming contradictory statement can be appreciated upon considering the mechanism of monosomic rescue after mostly maternal nondisjunction, this being the usual initiating cause of UPDpat.

Rare mechanisms to generate complete UPD include the following:

- Correction of interchange trisomy
- Correction of interchange monosomy
- Isochromosome formation
- Correction of imbalance due to small marker chromosome

If one parent carries a reciprocal translocation, asymmetric segregation of the chromosomes may lead to an interchange trisomy (p. 89) at conception, in which the translocation chromosomes, plus one of the normal homologs, are transmitted. Postzygotic correction by the loss of one homolog restores disomy, but if it is the other parent's chromosome that is lost, UPD is the consequence. Or, if a nullisomic gamete meets a normal gamete (interchange monosomy), the normal gamete may replicate the homolog in question, to restore disomy (just as in monosomy rescue, mentioned earlier).

Kotzot (2001) recorded 22 examples of UPD associated with a reciprocal or Robertsonian translocation, involving UPDs for chromosomes 7, 13, 14, 15, and 16. In the case of parent with a Robertsonian translocation, the most common mechanism leading to UPD is a trisomy rescue after nondisjunction. A monosomic acrocentric chromosome, after nondisjunction from a Robertsonian translocation parent and fertilization with a normal gamete, could replicate as an isochromosome in a monosomy rescue (Berend et al., 2000a; McGowan et al., 2002). Complementary isochromosomes (p. 157), of which scarcely a double-digit number have ever been described, can even allow the circumstance of “contraposed UPD”: that is, there may be UPD of the p arm from one parent, and UPD of the q arm from the other. Finally, in the setting of a supernumerary small marker chromosome (SMC), there may be a coexisting UPD for the same chromosome from which the SMC was derived (James et al., 1995; Liehr, 2010).

Segmental Uniparental Disomy

Segmental UPD may arise as the consequence of a postzygotic somatic recombination, between the maternal and paternal homolog (Fig. **22–3**), and in that case it will necessarily be an isodisomy (Kotzot, 2008a). An assessment of “long contiguous stretches of homozygosity” may prove a useful means to demonstrate the state (Papenhausen et al., 2011). The UPD segment lies distally, the rest of the chromosome having a normal biparental disomy. The classical karyotype is normal. An alternative mechanism is the following sequence: meiotic nondisjunction producing a disomic gamete, a trisomic conception, a mitotic crossing-over between a maternal and a paternal chromatid, and finally loss of one of the chromosomes that had come with the disomic gamete. UPD can have an effect if the particular chromosomal segment incorporates loci subject to imprinting. If the recombination occurs in a cell after the formation of the inner cell mass (which gives rise to the embryo), the segmental UPD will involve only some cells; in other words, there is mosaic segmental UPD. Beckwith-Wiedemann syndrome, Russell-Silver syndrome, UPDs for chromosome 14, and transient neonatal diabetes mellitus are conditions in which segmental UPD may apply.

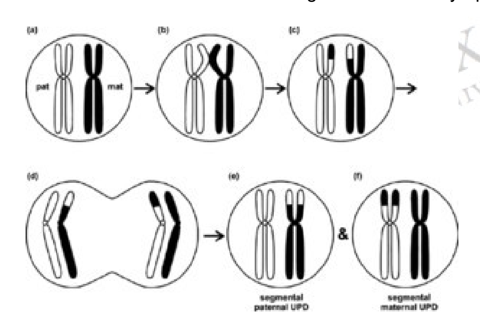


Figure 22–3
A mechanism whereby segmental uniparental (iso)disomy may be generated. In one cell of the early conceptus, the paternal and maternal homologs of a chromosome pair (*a*) undergo somatic recombination between the short arms (*b* and *c*). Segregation at mitosis (*d*) produces daughter cells with segmental uniparental disomy (UPD): in one (*e*), the short arm distal segments of both chromosomes are now of paternal origin, and in the other (*f*), they are both of maternal origin. These cells can then be the source of segmentally UPD tissue in a part of the conceptus.[9]

If a segment subject to UPID, due to one of the aforementioned scenarios, contains a recessive mutation, the disease related to that mutation will result. An example is given in Wattanasirichaigoon et al. (2008), who describe a woman heterozygous for the common Southeast Asian α-globin deletion on chromosome 16p, and who had a fetus with hemoglobin Bart's hydrops, a lethal disease. The likely scenario is that the ovum contained two copies of chromosome 16, which were isodisomic for distal 16p due to meiosis I recombination. Fertilization led to trisomy 16, but with the paternal homolog then lost by "rescue." Thus, the resultant fetal genotype was homozygosity for the α-globin deletion.

A partial trisomy might have different abnormal phenotypic effects according to the parental origin of the duplicated segment, if that segment is subject to imprinting. Trisomy for distal 14q provides an example. A similar picture of dysmorphology and psychomotor deficit is seen in either paternally or maternally originating 14q trisomy. But low birth weight, sometimes less than 2000 grams for a full-term baby, is a specific observation when the duplicated 14q segment comes from the mother (Georgiades et al., 1998). A classic example is the dup15q13, described in detail on p. 329: inherited from the father, there is typically no phenotypic consequence, but when the duplication is transmitted maternally, the child develops autism.

Aberrant Imprinting in a Biparental Setting.

A chromosomal segment that is normally imprinted (thus, inactive) may lose its imprint and become active. This is "relaxation" (or inappropriate erasure) of the imprint effect, and it may be termed an "epimutation"; to reemphasize the point, the DNA sequence remains unchanged. Consider Beckwith-Wiedemann syndrome (BWS). In some BWS with normal biparental inheritance of chromosome 11, the *IGF-2* (insulin-like growth factor 2) and *KCNQ1OT1* loci on distal 11p show biallelic expression; normally, only the paternal alleles should be functional. This overexpression of genes contributes to the overgrowth that is characteristic of the syndrome (as discussed in more detail later). An iatrogenic cause of aberrant imprinting may relate to pregnancy following assisted reproductive technology; aspects of the process of artificial ovulation stimulation, or of the embryo's environment in vitro, may disturb DNA methylation (Kagami et al., 2007; Amor and Halliday, 2008; Khoureiry et al., 2008; Marques et al., 2008; Katari et al., 2009).

Uniparental Disomy Phenotypes

Uniparental disomy has been observed for every chromosome except for 19 (Liehr, 2010). For most chromosomes, there is no apparent phenotypic consequence. For others, there may be, and we list below some of the proposed syndromes of UPD. The reader seeking more detail is referred to Engel and Antonarakis (2002), Kotzot (2008a), and Amor and Halliday (2008). Some conditions in which UPD had been thought of as a fair possibility have turned out not to be: Cornelia de Lange syndrome and Sotos syndrome, for example. In the case of UPD arising from incomplete trisomic rescue, additional factors of trisomy of the placenta, and/or a residual low-level trisomy of the fetus, may also contribute to the eventual phenotype. De Pater et al. (1997) note that a fetal trisomic cell line may not be detected unless the possibility of mosaicism is painstakingly pursued, and Benn (1998) uses the expression "occult mosaicism" to denote an unprovable suspicion. Because mosaicism can never be completely excluded, and neither can homozygosity for an unknown recessive mutation, one should generally incline in the direction of accepting that there is an absence of any UPD effect, when instances are known both of normal and of abnormal phenotypes, or when the observed abnormalities are inconsistent (Kotzot, 1999). The abnormal phenotypes will more likely be due to non-UPD mechanisms.

Certain clinical groups might be considered as candidates to harbor cases of UPD. Intrauterine growth retardation (IUGR) is an obvious category. Moore et al. (1997) addressed the question in a study of a cohort of 35 severely affected babies with a 46,N karyotype. Two instances of UPD (5% of the total) were identified, both with upd(16), and in each a coexisting placental 47,+16/46 mosaicism was shown. Neither baby survived. Kotzot et al. (2000a), checking chromosomes 2, 6, 14, 16, 20, and 22, found no instances of UPD in a series of 23 cases of IUGR, using a broader definition of birth weight and/or length below the 10th centile (10 of the 23 were considered to have Silver-Russell syndrome). Eggermann et al. (2001) studied 21 patients with pre- and postnatal growth retardation, choosing chromosomes 2, 7, 9, 14, 16, and 20 for analysis, and identified one with upd(14)mat and one with upd(20)mat. Except for the upd(20)mat, which may or may not have been the cause of the child's "minor features," it is to be noted that only already known UPDs (for 7, 14, 15, and 16) were identified in these several surveys.

"Unclassified congenital developmental defects" was the criterion for entry to the study of Ginsburg et al. (2000), comprising a cohort of 50 individuals, whose mothers had been aged 35 years or older at the time of delivery. This sort of patient is, of course, very familiar to the genetic counselor. Four turned out to have a UPD. The specific UPDs were as follows: maternal heterodisomy 14 in a woman with short stature and early puberty; paternal isodisomy 15 in a boy with previously undiagnosed (but retrospectively apparent) Angelman syndrome; upd(16)mat along with a partial 16p trisomy; and a child with, in retrospect, Silver-Russell syndrome, having a upd(7)mat. Four out of fifty (8%) may be a higher fraction than could usually be expected, and it is easy to be wise after the event that two of the cases were not really "unclassifiable." A lower fraction, namely 0%, was observed in a larger study of 120 children with two or more malformations, developmental or growth retardation, and a normal routine karyotype (Rosenberg et al., 2001).

Concerning a possible contribution to spontaneous abortion, Shaffer et al. (1998) studied 18 cases of cytogenetically normal first-trimester miscarriages, with every chromosome analyzed, but none showed UPD. In a more extensive study, Fritz et al. (2001) analyzed products of conception along with parental blood samples, which included some 77 spontaneous abortions with normal karyotypes. Of these, only two showed UPD: one case of maternal UPHD 9, and one of paternal UPID 21. Thus, from these two reports, only 2% of karyotypically normal spontaneous abortion is associated (whether or not this be causally) with UPD.

We have already noted the UPD effect of reduction to homozygosity of a recessive mutation. Engel and Antonarakis (2002) list 22 reports in which this sort of mechanism has been identified, including one notable example in which a child had both cystic fibrosis and Kartagener syndrome, the loci for these two separate recessive disorders lying on chromosome 7. The most extraordinary case is that of a couple, both normal homozygotes, whose child had maple syrup urine disease due to fresh mutation in oögenesis, with meiosis II nondisjunction then producing an isodisomic ovum (Lebo et al., 2000).

We list, by individual chromosome, the UPD syndromes, or associations with normality, that are on record. In each, the additional comment can be made, as just mentioned, that UPID could lead to unmasking of a recessive disorder; and likewise that an undetected residual trisomy might contribute to a phenotype, when the UPD mechanism has been trisomy correction.

Chromosome 1.

Maternal UPD of chromosome 1 may have of itself no effect (provided no recessive mutations are unmasked, as exemplified in Miura et al., 2000, and illustrated in Fig. **22–1b**). Field et al. (1998) made the serendipitous discovery of UPD 1 in a normal diabetic adult in the course of a genetic study of diabetes, as did Miyoshi et al. (2001) in their investigation of two normal persons with anomalous Rh blood grouping results: upd(1)mat in the former, mosaicism for paternal isodisomy 1 in the latter. Unmasking of recessive genes, rather than an effect of imprinting, may have been the basis of phenotypic abnormality in a unique case of upd(1)pat described in Chen et al. (1999c). A woman of normal intelligence had a myopathy, short stature, sterility, and deafness. In this case, there was a paternal isodisomy, with the chromosome 1 elements present in the form of two isochromosomes, i(1)(p10) and i(1)(q10).

Chromosome 2.

It is yet unclear whether a maternal UPD2 syndrome exists (Shaffer et al., 1997b; Wolstenholme et al., 2001b). In five patients, the recurrent observations included intrauterine and postnatal growth retardation (four of five cases), atypical bronchopulmonary dysplasia/hypoplasia (three cases), and hypospadias (two cases). Isozygosity for a recessive mutation, in this case the *ABCA12* gene located at 2q34 that is the basis of severe harlequin ichthyosis, was the result of trisomic rescue in a case reported by Castiglia et al. (2009), an interpretation underpinned by the observation of nonmosaic trisomy 2 at chorionic villus sampling.

Chromosome 3.

There is a single case from the earlier literature (Betz et al., 1974) of a retarded girl homozygous for a rare cytogenetic polymorphism, carried by only one parent, which might possibly be an example of upd(3).

Chromosome 4.

UPD4mat, isodisomic or heterodisomic, may be without a phenotype per se, the inconsistent abnormalities in the cases studied (three iso-, one heterodisomic) conceivably having some other cause (Kuchinka et al., 2001; Middleton et al., 2006). Cottrell et al. (2011) report a case of (autosomal recessive) limb girdle muscular dystrophy type E, for which the suggested sequence of events was as follows: mother heterozygous for recessive mutation; advanced maternal age; aberrant recombination between chromosome 4 homologs at maternal meiosis; meiotic nondisjunction; trisomy 4 conception; trisomy rescue; maternal UPD 4; isozygosity of the causative gene.

Chromosome 5.

Paternal UPD5 may be of no effect (Engel and Antonarakis, 2002).

Chromosome 6.

The defining feature of transient neonatal diabetes mellitus (TNDM) is hyperglycemia requiring treatment with insulin, with a gradual resolution to normal glucose metabolism in the first few months of life, although with a risk subsequently for non-insulin-dependent diabetes in adult life. In at least a substantial fraction of cases, TNDM is due to upd(6)pat, with 6q22-q24 being the crucial region. A methylation defect within the critical region, or 6q22–q24 duplication of paternal origin, this latter accounting for all familial cases, are other routes whereby TNDM may arise (Temple and Shield, 2002). One example due to a familial insertion involving the segment 6q22-q23 is mentioned on p. 237. Parker et al. (2006) describe a child with congenital adrenal hyperplasia (the 21-hydroxylase gene being on chromosome 6) and Klinefelter syndrome, 48,XXY,+mar(6), with maternal isodisomy for both chromosomes 6 and X. “Correction” of fetal trisomy 6 was the probable basis of upd(6)mat identified by Cockwell et al. (2006) in a case of fetal death in utero. But upd(6)pat can also be without apparent effect, as witness an otherwise normal girl with thalassemia whose family was being studied to find a donor for marrow transplantation, and who turned out to have paternal UPID 6 (Bittencourt et al., 1997).

Chromosome 7.

Silver-Russell syndrome (SRS) has as its major feature intra-uterine and postnatal growth retardation, often with associated limb asymmetry. Genetic causes include maternal UPD 7 (about 10%), and 11p15 epimutation and structural 11p aberrations (see section on “Chromosome 11”), with clinical and endocrine observations differing somewhat in each type (Binder et al., 2008; Wakeling et al., 2010). There is a maternal age association: very few SRS children born to mothers under age 35 have UPD 7, but around half of those born to mothers 35 or over are due to upd(7)mat, and this may reflect a maternal meiosis I error as the underlying cause (Ginsburg et al., 2000). One case is recorded of SRS in the setting of a maternal reciprocal translocation involving chromosome 7: the conception was probably an interchange trisomy, 47,t(7;16)(q21;q24),+7, with subsequent loss of the paternal chromosome 7 producing the balanced state, but with a maternal UPHD 7 (Dupont et al., 2002).

As for paternal UPID 7, only two cases have been discovered (Engel and Antonarakis, 2002). One was a woman of normal linear growth, and a normal intellect, and it was only because she had a recessive condition with its locus on chromosome 7 (congenital chloride diarrhea) that she had been investigated (Höglund et al., 1994).

Chromosome 8.

UPID 8 (pat) is apparently without any phenotypic effect, and one may suppose that this reflects a lack of imprinted genes on this chromosome. Benlian et al. (1996) had made the fortuitous discovery in an otherwise normal child with lipoprotein lipase deficiency, a recessive condition for which the locus maps to 8p22. Similarly, Karanjawala et al. (2000) discovered maternal isodisomy 8 by chance in a man participating in a diabetes research study. He was himself nondiabetic, although he did have the unusual history of a neuroendocrine gut tumor (carcinoid) at atypically young age.

Chromosome 9.

Maternal UPD 9 appears to be without effect (Björck et al., 1999; Engel and Antonarakis, 2002). Homozygosity at the *SURF-1* locus due to isodisomy 9 is documented in twins with Leigh syndrome (Tiranti et al., 1999).

Chromosome 10.

Maternal UPD 10 appears to be without effect (Jones et al., 1995). In uphd(10)mat with concomitant trisomy 10 mosaicism, it is presumably the trisomy rather than the UPD that causes a severe phenotype (Hahnemann et al., 2005).

Chromosome 11.

There are growth regulation loci in 11p15 that are expressed monoallelically, according to the parent of origin of the allele. These include the paternally expressed genes *IGF2* and *KCNQ1OT1*, and the maternally expressed genes *H19* and *CDKN1C*. *IGF2* and *H19* are located within one of two “differentially methylated regions”[4] (DMR1), such that *IGF2* is only expressed from the paternal allele, and *H19* only from the maternal allele. Similarly, *KCNQ1OT1* (paternal expression) and *CDKN1C* (maternal expression) are under the control of the second region, DMR2 (Weksberg et al., 2010; Manipalviratn et al., 2009). Perturbation of these regions and genes can lead to two syndromes of opposite growth disorder: Beckwith-Wiedemann syndrome (BWS), of which overgrowth and hemihyperplasia are characteristic, and Silver-Russell syndrome (SRS), in which growth retardation and hemihypoplasia are key features.

Beckwith-Wiedemann Syndrome.

Mosaic segmental upd(11p)pat is the basis of about 20% of sporadically occurring BWS. In BWS, the striking clinical picture is that of overgrowth of tissues and organs. Thus, in upd(11p)pat, *IGF2* and *KCNQ1OT1* are expressed biallelically; and *H19* and *CDKN1C* are silenced (“nulliallelic”). This imbalance is the basis of the excessive growth. Hemihyperplasia is a clinical indicator of this category, and those tissues with the greater fraction of upd(11p) cells may show a greater degree of overgrowth. Itoh et al. (2000) describe a child with BWS having a normal adrenal gland on the right and a very enlarged one on the left: 30% of cells in the right gland had upd(11)pat, compared with 88% on the left. Epigenetic mechanisms exist due to other than UPD, noted in the later section on “Genetic Counseling” and as outlined in Figure **22–4** and Table 22–1. BWS due to 11p15 epimutation, affecting in particular the DMR2, has a particular association with IVF (Amor and Halliday, 2008; Lim et al., 2009; Manipalviratn et al., 2009).

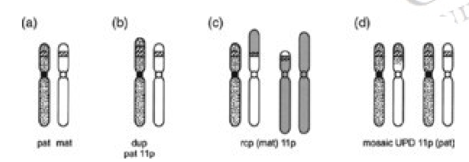


Figure 22–4
The no. 11 chromosomes in different chromosomal bases of Beckwith-Wiedemann syndrome (BWS). The maternal homolog is shown open, the paternal homolog is speckled, and the BWS critical region at 11p15 is shown cross-hatched. (*a*) The normal state of biparental inheritance of intact no. 15 chromosomes. (*b*) Paternal duplication of distal 11p. (*c*) Maternal reciprocal translocation disrupting the BWS critical region, with the other chromosome shown gray. (*d*) Mosaic segmental paternal uniparental disomy (UPD) of 11p, showing the chromosome 11 pairs of the two cell lines. The pair on the left shows paternal UPD for distal 11p (the speckled segments).

Table 22–1. Different Causes of Beckwith-Wiedemann and Silver-Russell Syndromes (see also Fig. 22–4)

	FRACTIONS (%)	
GENETIC FORM	**BWS**	**SRS**
Epigenetic Error on 11p15:		
Gain/loss of methylation at DMR1	5 (gain)	50 (loss)
Loss of methylation at DMR2	50	
Uniparental disomy	20 (upd11pat)	5–10 (upd7mat)
Chromosomal 11 Alterations:		
Duplication	< 1 (pat)	1–2 (mat)
Paternal microduplication	<<1	
Maternal microduplication		<<1
Maternal microdeletion	<<1	
Inversion, translocation	< 1	<<1
CDKN1C mutation	10	
Unknown	10	40

Note: Fractions (rounded) indicate relative frequencies; these data may be influenced by the clinical index of suspicion.

DMR1 and 2, differentially methylated regions 1 and 2. DMR1 gain of methylation causes overexpression of *IGF2* and nonexpression of *H19*. DMR2 loss of methylation causes overexpression of *KCNQ1OT1* and nonexpression of *CDKN1C* (and see text).

Sources: Algar et al., 2007; Manipalviratn et al., 2009; Eggermann et al., 2010a; Wakeling et al., 2010; Zollino et al., 2010a.

Paternal UPD 11 for larger extents of chromosome 11, and maximally the whole chromosome, may lead to typical or to more severe forms of BWS, or to a phenotype with severe intrauterine growth retardation, the differences likely reflecting tissue distribution of the UPD lineage (Grati et al., 2007).

Silver-Russell Syndrome.

SRS due to 11p anomaly can be considered the countertype to BWS, both clinically and at the molecular level (Schönherr et al., 2007). In SRS due to upd(11p)mat, or to 11p “epimutation” (hypomethylation of DMR1), the maternally active gene *H19* functions biallelically, whereas *IGF2* is underexpressed (Horike et al., 2009). Isolated hemihypoplasia, with shorter limbs on one side, has been recorded in association with epimutation (Zeschnigk et al., 2008). SRS can also be due to upd(7)mat, as noted earlier; the two genetic forms have different underlying causes of the growth retardation (Binder et al., 2008).

Wilms Tumor.

In a study of 437 (nonsyndromic) Wilms tumor patients, Scott et al. (2008a) showed, in 13 of them, 11p15 abnormalities of the same sort that may be seen in BWS: upd(11p), and epimutations, a microinsertion, and a microdeletion in DMR1. A sibling of the child with a maternally inherited DMR1 microdeletion had a clinical diagnosis of BWS, illustrating that the identical genetic factor, although presumably with differing levels and distribution of postzygotically arising mosaicism, can underlie the two disorders.[5]

Chromosome 13.

Neither maternal nor paternal UPD 13, iso- or heterodisomy, appears to have any effect upon the phenotype (Berend et al., 1999; Soler et al., 2000). A unique example of familial UPD13, paternal and maternal, emphasizes this point: a normal mother with presumed 45,XX,i(13q)pat had a normal child with 45,XY,i(13q)mat (Slater et al., 1995). She may have been the result of monosomic rescue, and her son due to trisomic rescue!

Chromosome 14.

UPD 14 produces different syndromes according to the paternal or maternal basis of the disomy (Sutton and Shaffer, 2000; Engel and Antonarakis, 2002). Either may be seen in the setting of a normal karyotype or with a Robertsonian translocation (or “acrocentric isochromosome”). A balanced 45,der(13;14) Robertsonian translocation may reflect correction of an initially 46,der(13;14), +14 conception, while the 45,der(14;14) case might in fact result from a 45,–14 conception which then corrected by reduplication of the single chromosome 14 to give an i(14q) with isodisomy. Isodisomy may occur in the setting of a normal karyotype, and it may thus be less rare than is appreciated (Chu et al., 2004). *Paternal* UPD 14 is the more severe, with obstetric complication (polyhydramnios and premature labor), a particular pattern of malformation, growth retardation, and major functional neurological compromise (Sutton et al., 2003; Stevenson et al., 2004). Survival is poor. The bell-shaped thorax, reminiscent of Jeune syndrome, is a particular clinical pointer, and it has been observed at 23-week ultrasonography (Curtis et al., 2006); this anatomy may improve during childhood in those who survive (Chu et al., 2004; Kagami et al., 2005). *Maternal* UPD 14 produces a syndrome of pre- and postnatal growth retardation, a characteristic facies (to which the effects of an arrested hydrocephalus may contribute), and intellectual development may be “low-normal to normal.” Mitter et al. (2006) point out the overlap, to some extent, with the Prader-Willi neonatal phenotype.

The critical segment, wherein the imprintable loci reside, is 14q32.2; the clinical picture is essentially similar in those with full or segmental UPD14 (Irving et al., 2010), and indeed the phenotype is also similar in cases with an epimutation (recognizable due to a marked hypomethylation at key loci within 14q32.2) (Zechner et al., 2009).

Chromosome 15.

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are the two UPD15 syndromes. It may be an oversimplification, but equally it may be a valid perspective, to

think of these as each being caused by absent activity of a single genetic segment, or of a single gene, within 15q11–13. In PWS, the basic defect may be absent activity of a transcript on the *paternal* chromosome 15, and which codes for a particular cluster (HBII-85) of small nucleolar RNAs (snoRNA) (Sahoo et al., 2008; de Smith et al., 2009). Different components of the PWS phenotype might therefore be mediated via perturbed functioning of different genetic targets of these snoRNAs. AS is due to absent activity of the *UBE3A* gene on the *maternal* chromosome 15. HBII-85 and *UBE3A* lie in close proximity on 15q11-q13, and both are under the influence of an imprinting control center (IC): from centromeric to telomeric on the chromosome, the order is IC, the HBII-85 cluster, and the *UBE3A* gene.

The absence of activity is due either to the loss, or to the nonfunctioning, of this PWS/AS region on one chromosome 15 homolog. *Loss* is most commonly due to a simple interstitial deletion ("classical deletion"). Low-copy repeats on either side of the region can come together and set the stage for nonallelic homologous recombination (p. 296), leading to deletion of the PWS/AS region. Whether the phenotype comes to be PWS or AS depends upon which parent contributed the deleted chromosome. *Nonfunctioning* of (structurally normal) genes within 15q11q13 is due to the imprint status. This is most commonly the consequence of UPD 15, with the phenotype determined according to the parent of origin of the disomic pair of chromosomes. A rare cause is failure of, or damage to, the chromosome 15 IC. Study of these IC-damaged cases has cast much light on the processes of molecular pathogenesis in PWS and AS, and so the length of their commentaries that follow is quite out of proportion to their frequencies. In the case of AS, *mutation* in the *UBE3A* gene is a further category of mechanism.

The 15q11q13 Imprinting Center.

Normal persons have one paternally imprinted chromosome 15 and one maternally imprinted chromosome 15. The imprinting state of a chromosome 15 is set and reset as it is transmitted down the generations, according to the sex of the transmitting parent. This resetting—an "epigenetic modification"—is dictated during gametogenesis from the *cis*-acting IC, with methylation of genes comprising, in large part at least, the crux of the process. The IC is bipartite, with a centromeric element, the AS-IC, and 35 kb distant a telomeric element, the PWS-IC, this latter including exon 1 of *SNRPN*. Interaction between these two elements directs the process. In *maternal* gametogenesis, the AS-IC has responsibility for initiating a paternal→maternal switch on the chromosome 15 that the mother herself had received from her father. The chromosome 15 she got from her mother retains a maternal imprint. With an active AS-IC, the *UBE3A* gene, lying about 1 Mb distant, is free to function, in the embryo to which this ovum gives rise. Vice versa, *paternal* gametogenesis serves to effect a maternal→paternal switch, or to retain a paternal status, on the chromosome 15 that the sperm contributes to the embryo. In consequence, a number of genes under its aegis are able to function, in part at least by being demethylated. The *UBE3A* gene's activity is prevented. These epigenetic modifications operate only in *cis*, and so the maternal and paternal chromosomes continue to function autonomously, with their different repertoires of expression, during the life of the individual.

A scheme for the various molecular defects of PWS and AS is presented in Figure **22–5**; Horsthemke and Wagstaff (2008) provide a review. Table 22–2 sets out the test results for the different types of PWS and AS.

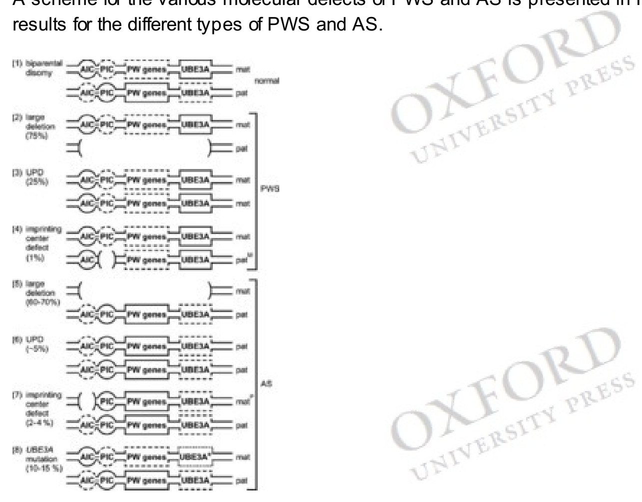




Figure 22–5
An outline of the different genetic forms of Prader-Willi syndrome (PWS) and Angelman syndrome (AS). The PWS/AS critical region of chromosome 15 is depicted. A bipartite imprinting center with AS and PWS components (AIC and PIC) controls, in *cis*, the activity of a set of PWS genes and the *UBE3A* gene. A switched-on IC and an actively functioning gene are shown in unbroken line; a switched-off IC and an unactivated gene are shown in dashed outline. A mutated *UBE3A* gene is shown starred and with a dotted outline. (1) Normally, the *UBE3A* gene is transcribed only from the maternal chromosome (mat), and the PWS genes only from the paternal chromosome (pat), with each chromosome thus functioning appropriately for its parent of origin. In PWS there is nonfunctioning of the PWS genes because: (2) the PWS genes have been removed by a typical large deletion from the paternal chromosome; (3) both chromosomes are of maternal origin; (4) a microdeletion of, or mutation in, the PIC has fixed a maternal imprint status on the paternal chromosome. In AS there is nonfunctioning of the *UBE3A* gene because: (5) the *UBE3A* gene has been removed by a typical large deletion from the maternal chromosome; (6) both chromosomes are of paternal origin; (7) a microdeletion of, or mutation in, the AIC has fixed a paternal imprint status on the maternal chromosome; (8) there is a mutation in the *UBE3A* gene on the maternal chromosome. A further category (9) is not shown, comprising the 10%–15% in which no genetic defect can be shown. Approximate percentages of each PWS/AS category are indicated; in another ~10% of AS no genetic defect can be identified. patM, a maternally functioning chromosome of paternal origin; matP, a paternally functioning chromosome of maternal origin.

Table 22–2. Assessment of Genetic Category of Prader-Willi and Angelman Syndromes According to Results of Cytogenetic and Molecular Testing

				METHYLATION		
		CYTOGENETICS	FISH	PATTERN OF NO. 15s	PARENTAL ORIGINS OF NO. 15s	*UBE3A* GENE
Prader-Willi syndrome	Classical deletion	±	+	mat	bi	
	upd(15)mat	N	N	mat	mat	
	Imprinting center	N	±	mat	bi	
		microdeletion				
	Other imprinting	N	N	mat	bi	
	center defect					
Angelman syndrome	Classical deletion	±	+	pat	bi	deleted
	upd(15)pat	N	N	pat	pat	“intact”
	Imprinting defect	N	N	pat	bi	“intact”
	UBE3A mutation	N	N	bi	bi	mutated
	Epigenetic error	N	N	bi	bi	“intact”

Notes: A normal cytogenetic/FISH result is indicated by N, and an abnormal result by +. Most classical deletions are detectable cytogenetically, but a few may be missed (indicated as ±). Methylation patterns/parental origins: FISH, fluorescence in situ hybridization; mat, maternal; pat, paternal; bi, biparental. “Intact” means that the DNA sequence of the gene is normal, but its function is epigenetically compromised.

Classical Deletion.

This is the most frequent basis of the two syndromes, accounting for about 70% of both PWS and AS (Horsthemke and Buiting, 2006). The deletion removes 5.9 (class I) or 5.0 (class II) Mb within 15q11q13, encompassing the PWS and the AS genetic elements, and including the IC. There is one common distal breakpoint (BP3), and two variable proximal deletion breakpoint regions (BP1, BP2), due to duplicons at these sites. Nonallelic homologous recombination between the distal, and whichever proximal duplicon, then causes the deletions (Ji et al., 2000). The behavioral phenotype is a little worse with the class I BP1-BP3 deletion than the class II BP2-BP3 deletion (Bittel et al., 2006). Larger deletions are infrequent and are associated with a more severe phenotype (Sahoo et al., 2007).

If the deletion occurs on a *paternally* originating chromosome, it will cause the PWS phenotype to develop[6]; and vice versa, a *maternal* deletion produces AS. In a sense, there is an “unmasking of the silent elements” on the other chromosome. As well as the crucial PWS and AS genetic elements, a number of other loci may be deleted, and so the expression “contiguous gene syndrome” is not inappropriate, albeit having a somewhat different sense from its usage elsewhere in this book. One of the least important of these other loci is the *P* gene that contributes to normal pigmentation, and so children with PWS and AS due to classical deletion typically have fairer complexions than do their siblings.[7] Mosaicism may lead to a milder phenotype (Golden et al., 1999; Tekin et al., 2000). In very rare cases of PWS with concomitant 47,XXY Klinefelter syndrome, the coincidence of the two conditions is likely to be fortuitous (Nowaczyk et al., 2004).

Prader-Willi and Angelman Syndromes due to Deletion, Associated with Uncommon Rearrangement.

Loss of the PW/AS region can be due to transmission of an unbalanced translocation or an inversion involving chromosome 15. The male carrier of a balanced reciprocal translocation in which one breakpoint is in the region of 15q13 can transmit an unbalanced complement to produce a deletion PWS child (Hultén et al., 1991; Smeets et al., 1992), and the female carrier can have a child with deletion AS (Stalker and Williams, 1998). There may be an additional effect from the concomitant imbalance involving the other chromosome of a translocation, such as the case in Torisu et al. (2004), a child who displayed features both of Angelman syndrome and the 1p36 deletion syndrome, due to a tertiary monosomy for these two segments, the mother being a balanced translocation carrier. A handful of PWS cases have been due to a Y;15 translocation with breakpoints in Yp and at 15q12-q13, deleting the PWS region, having the karyotype 45,X,der(Y),t(Y;15) (Vickers et al., 1994). A grandmother heterozygous for an inverted insertion of chromosome 15 had a PWS grandchild through her carrier son and an AS grandchild through her carrier daughter (Collinson et al., 2004).

Uniparental Disomy and Prader-Willi Syndrome.

About 30% of PWS is due to UPD (Horsthemke and Buiting, 2006). The cytogenetic study typically shows a normal 46,XX or 46,XY karyotype. Both chromosomes 15 come from the *mother*, and so neither of the PWS critical regions is expressed. This functional lack causes the PWS phenotype. In most (80% or more), the UPD had its origin in a maternal meiosis I nondisjunction. A maternal age effect is clear: five times as many PWS children born to mothers under age 35 have a deletion as have UPD, but the reverse applies to those born to mothers 35 or over, in whom there is a five-fold excess of those showing UPD (Ginsburg et al., 2000). The phenotype is very similar to classical deletion PWS, although the facies may be less “typical” with the UPD form of PWS, and some of the minor manifestations are less likely to occur; in consequence, diagnosis may be delayed in comparison to deletion PWS (Cassidy et al., 1997; Gunay-Aygun et al., 1997). The UPD form of PWS is particularly associated with a psychiatric phenotype, typically presenting in young adulthood and characterized by a fluctuating psychosis and bipolar mood disorder (Verhoeven et al., 2003). A more severe form of UPD PWS is associated with a concomitant trisomy 15 mosaicism (Olander et al., 2000).

Uniparental Disomy and Angelman Syndrome.

Only about 1% of AS is due to UPD (Horsthemke and Buiting, 2006). As with PWS due to UPD, the karyotype is normal 46,XX or 46,XY. Both chromosomes 15 are from the *father*, and neither chromosome expresses the AS critical region. Most cases involve a postzygotic origin of the extra paternal chromosome, possibly following the “correction” of monosomy 15 due to a nullisomic ovum (as outlined earlier), and as with PWS, this is likely to reflect a maternal age effect. Very few AS children born to mothers under age 35 have UPD, but those born to mothers 35 or over have about equal numbers due to deletion and UPD (Ginsburg et al., 2000). A few are due to a paternal second meiotic error (Robinson et al., 2000). In parallel with the observations in UPD PWS noted earlier, the phenotype in AS due to UPD is not quite as severe as in the deletion form, with these children showing a lesser frequency of seizures, and some having a few words (Fridman et al., 2000). But it remains true that the handicap is severe.

Prader-Willi and Angelman Syndromes Due to Uniparental Disomy, Associated with Chromosome 15 Rearrangement.

Uniparental disomy can result from a variety of rearrangements involving chromosome 15. The male carrier of a reciprocal translocation involving chromosome 15 could transmit a disomic 15 spermatocyte from 3:1 nondisjunction, with the maternal chromosome 15 then being lost, and have a child with UPD AS; and vice versa, the female carrier could have a PWS child (Calounova et al., 2006; Heidemann et al., 2010). Similarly, a familial nonhomologous Robertsonian translocation in which one of the component chromosomes is a no. 15 giving a trisomic 15 conception, and with postzygotic loss of the chromosome 15 from the other parent, would lead to upd(15) with either PWS or AS, according to the sex of the carrier parent (Fig. 7–6) (Tsai et al., 2004). The same thing could happen if the translocation were de novo. A maternally originating de novo homologous der(15;15) (which may actually be a 15q isochromosome), with no chromosome 15 contributed from the father, would cause PWS (Robinson et al., 1994); and, vice versa, AS would result from a paternal isochromosome 15q (Tonk et al., 1996). Smith et al. (1994) describe AS from asymmetric segregation of a paternal 8;15 translocation (Fig. 8–5). The heterozygous father passed on his der(8) and his normal chromosome 15 (thus, paternal UPD), and there was absence of a maternal chromosome 15. Some PWS children with a 47,+idic(15) karyotype may actually have UPD of the two intact chromosomes 15, and the idic (15) is a phenotypically irrelevant relic of the original process of abnormal chromosomal behavior (Robinson et al., 1993).

Imprinting Center Defects.

A very small group of PWS and AS patients, about 1% and about 4%, respectively, have normal biparental inheritance and no classical deletion, but a uniparental pattern of methylation and gene expression (Horsthemke and Buiting, 2006). Most of these cases reflect *abnormal function* of the IC, while a minority, about 10%–15%, have an actual IC *microdeletion*. The latter category can be strongly suspected when there is a positive family history, while in the former, sporadic occurrence has been universally observed. Whether PWS or AS is seen depends upon which component of the IC is deleted or nonfunctional.

Functional Imprinting Center Defect.

Buiting et al. (2003) analyzed 44 PWS and 76 AS patients with a failure of IC functioning, an IC deletion or point mutation having been excluded; these aberrant epigenetic states are referred to as epimutations.[8] All cases were sporadic. Some shared with an unaffected sibling the 15q11-q13 haplotype on their paternal (PWS) or maternal (AS) chromosome, supporting the presumption of a de novo defect. With PWS, the basis of the epimutation may be a failure to erase the maternal imprint, as an act of omission (Buiting et al., 2003). Thus, for example, the father of such a PWS child passes on his maternal chromosome 15 with its maternal imprint still in place, and the child inherits two maternally imprinted no. 15 chromosomes. In AS, the typical scenario may be the imposition of an anomalous imprint status. This can be thought of as an act of commission: the mother inappropriately applies a paternal imprint to the chromosome 15 or fails to reset her paternal chromosome 15 that she passes to the child; or (since some maternal epimutations are mosaic) the error may occur postzygotically. If the error is incomplete, a milder AS phenotype may be seen (Brockmann et al., 2002).

AS due to an imprinting defect, with loss of methylation of the maternal allele, may have an association with subfertility and artificial reproductive technology (Manipalviratn et al., 2009). If the association is indeed causal, the biological basis may be in the subfertility per se, or due to the superovulation treatment as part of IVF protocol, which leads to a failure to acquire normal *UBE3A* activation status in the ovum.

Microdeletion of Imprinting Center.

Microdeletions of the IC, generally of kilobase size, remove one or other of its major component parts, either the PWS-IC or the AS-IC. The inability to reset an appropriate imprint status leads to the “fixation of an ancestral epigenotype” (Saitoh et al., 1997). Only a handful of cases have been identified worldwide (Horsthemke and Buiting, 2006). Their particular importance to the counselor lies in the high recurrence risk: the mode of inheritance is essentially sex influenced—the *parent's* sex, that is—autosomal dominant, with a 50% risk for the heterozygous father (for PWS) or the heterozygous mother (for AS), according to which component part of the IC is deleted. De novo mutations have been reported.

In *Prader-Willi syndrome due to IC microdeletion*, the father would have received the deletion on his mother's chromosome 15. He is normal, since an erased paternal imprint on his maternal chromosome is, naturally, correct. The deletion could have originated in his mother, or antecedent to her, provided transmission had been exclusively matrilineal. But when he passes this chromosome 15 with its fixed maternal epigenotype to a child of his, with the maternal→paternal imprint switch unable to function, the child has, effectively, a functional maternal UPD 15. Such a family is illustrated in Ming et al. (2000). Of 10 children, all of them normal and with normal karyotypes on standard cytogenetics, four inherited an IC microdeletion, presumably from their deceased mother (their father was proven not to have the deletion). Two of these children were male, and each went on to have, in the next generation, a child with PWS: an example of “grandmatrilineal inheritance.” The laboratory demonstration of an IC deletion is complex, in concept and in practice (Buiting et al., 2003).

In *Angelman syndrome due to IC microdeletion*, the scenario is essentially the obverse of the above. A microdeletion on the maternal chromosome 15 removes the AS-IC. The defect may have arisen de novo from the maternal grandfather of the AS child, or alternatively, there could have been patrilineal transmission of the mutation, harmlessly, for any number of previous generations. Transmission from the grandfather to the mother would be without phenotypic consequence, since a paternally originating chromosome 15 would in any event have its AS-IC inactivated. But in oögenesis in the mother, the normal paternal→maternal switch on the abnormal chromosome cannot be effected (thus, “fixation” of the ancestral paternal epigenotype). If the child receives this chromosome 15 from the mother, both homologs carry a paternal imprint. In consequence, the child has AS. Two such Japanese families, independently ascertained and reported, had exactly the same 1.487 Mb deletion, from nucleotides 22,938, 518 to 24,425,426, and may well have represented distant branches from the same, presumably male, ancestor (Sato et al., 2007).

Angelman Syndrome due to UBE3A Gene Mutation.

Classical point mutation, affecting the *UBE3A* (ubiquitin protein ligase 3A) gene, is an important contributor to AS etiology (Abaied et al., 2010). This gene is expressed from both parental chromosomes in some tissues, but, in the brain, from only the maternal chromosome. The (normal) paternal allele does not function in embryonic brain, or at least in particular parts of the brain. Thus, if the maternal gene is mutated, there is no *UBE3A* expression, and in consequence brain development is compromised (Rougeulle and Lalande, 1998). In a mouse knockout model, *Ube3a* expression was compromised in certain cells of the hippocampus, a crucial structure in learning and memory, and of the cerebellum, which may have a role in learning as well as its classic role in coordination (Albrecht et al., 1997). The human situation is quite likely to be similar. (Mouse knockout models for PWS are lethal.)

About 70% of inherited “nondeletion non-UPD non-IC” AS is due to *UBE3A* mutation of maternal origin. As for sporadic AS, and considering those patients with normal methylation results, a *UBE3A* mutation is seen in about 30%. The severity of phenotype in the mutation form falls between the deletion and UPD cases (Abaied et al., 2010). Multigenerational transmission may be seen, with the revealing observation that AS children are born only to carrier daughters of carrier males (Fig. **22–6**). The mutation transmitted by the father has no effect in his child, since this chromosome 15 region would in any event carry a paternal imprint and be silenced.

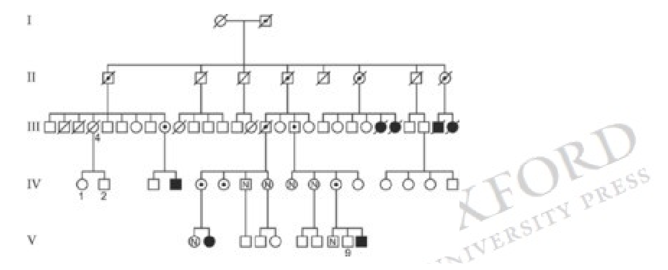




Figure 22–6
A family with inherited Angelman syndrome, due to a *UBE3A* mutation, reported in Moncla et al. (1999). Filled symbol, Angelman syndrome; bull's eye symbol, mutation carrier, demonstrated or inferred; N, demonstrated noncarrier. Note that all the affected children are born to carrier *mothers*, but that these mothers are related to each other through the *male* line. Some normal children have been proven to be noncarriers with molecular testing (N in symbol), but the reader can also determine that any unaffected child of a potential carrier mother, such as IV:1 and 2, the children of III:4, or V:9, the sibling of an affected child, cannot be carriers. An inherited imprinting center mutation could present a similar pedigree.

Angelman Syndrome with No Deletion, No Uniparental Disomy, No Imprinting Mutation, and No UBE3A Mutation

In some 15%–20% of AS, no genetic defect can be found ((Hitchins et al., 2004; Horsthemke and Buiting, 2006). There is a normal karyotype, with no deletion demonstrable on fluorescence in situ hybridization (FISH), normal methylation analysis (at least on the sampled tissues), biparental inheritance, and an apparently intact *UBE3A* gene. There may be an epigenetic influence whereby a normal *UBE3A* gene on the maternal chromosome fails to activate normally during embryogenesis. Or there may be some other AS genetic basis, as yet unknown.

Chromosome 16.

This is one of the more commonly seen UPDs, and it is almost always due to correction of trisomy 16 of maternal meiotic origin. Thus, it is typically a maternal UPHD. It has been difficult to separate out the effects of the UPD, and of a placental insufficiency due to confined placental mosaicism for trisomy 16, this typically being the route by which the UPD comes to be recognized, following chorionic villus sampling; and in addition, a possible residual occult fetal trisomy mosaicism always remains as a potential confounder. Yong et al. (2002) showed in a large series of mosaic trisomy 16 discovered at prenatal diagnosis, that the degree of fetal growth restriction, and probably the malformation rate, was greater in those with upd(16)mat than in those with biparental inheritance, thus suggesting a role of the UPD per se. A single case is recorded of body stalk defect in a fetus with upd(16)mat (Chan et al., 2000). As for paternal UPD 16, it seems probable that it has no clinical consequences (Engel and Antonarakis, 2002). The usual rare risk from isozygosity for a recessive gene applies, as exemplified in Wattanasirichaigoon et al. (2008), who report a child with hydrops fetalis due to hemoglobin Bart's, consequential upon upd(16)mat.

Chromosome 17.

Two cases of complete upd(17)mat have been described. One 46,XY child was normal, ascertainment having been via the discovery of trisomy 17 mosaicism at amniocentesis (Genuardi et al., 1999). Lebre et al. (2009) identified the UPD in an infant with cystinosis, a recessively inherited multiorgan storage disease, the locus of which is on chromosome 17p, and this segment being in isodisomic state in the child. The abnormal phenotype in a child with a segmental upd(17)mat, involving 17q25.3, may be due to some other factor (Rio et al., 2001).

Chromosome 20.

A prima facie case exists for a UPD 20 syndrome. Eggermann et al. (2001) review three reported cases of upd(20), one paternal and two maternal, with a major malformation phenotype in the former, and growth retardation the common observation in the latter two. A causal link is possible, as is also true concerning two abnormal children initially ascertained because of trisomy 20 mosaicism at amniocentesis (Salafsky et al., 2001; Velissariou et al., 2002). A single sporadic case of pseudohypoparathyroidism in association with a paternal upd(20)pat (UPD for the long arm) might reflect a true effect of the UPD, since the causative gene (*GNAS1*) on 20q is known to display a parent-of-origin effect (Bastepe, 2008). This abnormality probably arose as a mitotic event, according to the scenario as set out in Figure **22–3d**. Further support for this view comes from Aldred et al. (2002), who studied two children with deletions of 20q, one of maternal and the other of paternal origin, in whom the different patterns of parathyroid endocrinology were consistent with what is understood of the complex behavior of the *GNAS1* gene.

Chromosome 21.

UPD 21, maternal or paternal, appears to be without effect (Engel and Antonarakis, 2002).

Chromosome 22.

Maternal UPD 22 has generally not been causally associated with any defect (Kotzot, 1999; Engel and Antonarakis, 2002). Intrauterine growth retardation, if present, may more likely reflect the influence of a trisomic 22 placenta, or low-level “occult” mosaicism of the fetus (Balmer et al., 1999; Bryan et al., 2002). A child with UPD 22 due to “balancing” recombinant chromosomes 22 from parental inversions was developing normally at age 20 months (Kariminejad et al., 2011, and see p. [link]).

Chromosome X.

Neither upd(X)mat nor upd(X)pat appears to have any consequence in the 46,XX person, with the usual exception of homozygosity for a recessive mutation (Quan et al., 1997). However, there may be a subtly different neuropsychological phenotype according to the parent of origin in monosomy X (self-evidently a uniparental condition). In a British study, 80 girls with Turner syndrome underwent behavioral evaluation, 55 of whom were 45,X^M and 25 were 45,X^P. The 45,X^P girls were more socially adept and more articulate than the 45,X^M girls. Speculatively, this may represent the effect of an imprintable X-borne “locus for social cognition” that is functional on the X chromosome transmitted from a father, and nonfunctional on the X from a mother (Skuse et al., 1997). Autism, which is a male-susceptible condition, is associated with 45,X^M in the case of autistic girls with Turner syndrome (Donnelly et al., 2000). In terms of response to growth hormone, it makes no difference whether the child is 45,X^M or 45,X^P (Tsezou et al., 1999).

Upd(X)pat offers the intriguing scenario of father-to-son transmission of an X-linked gene. A 24,XY gamete from a hemizygous father could produce a 47,XXY zygote, which could subsequently lose the maternally contributed X; or the ovum could be nullisomic X, with sex chromosomal complementation producing 46,XY. Either mechanism could explain the observations in a family with the X-linked form of ectodermal dysplasia, as presented in Ferrier et al. (2009).

Uniparental Disomy for the Entire Chromosomal Complement (Complete Uniparental Disomy)

Nonmosaic paternal uniparental disomy (UPDpat) for the full diploid complement—all 46 chromosomes are of paternal origin—produces the placental disorder of complete hydatidiform mole. When, in addition to a double set of paternally derived chromosomes, there is also a haploid maternal set (triploidy, with a total chromosome count of 69), a partial hydatidiform mole results. Hydatidiform mole is discussed in more detail in Chapter 23.

UPDmat of an oöcyte, following failure of a premeiotic or of a meiotic cell division, leads to benign cystic ovarian teratoma, an unusual tumor of the ovary in which several embryonic tissues may be represented (Miura et al., 1999).

Mosaicism for Complete Uniparental Disomy.

Abnormal cytogenetic events around the time of fertilization—such as two sperm entering an ovum to produce a zygote with three pronuclei, or an ovum undergoing a mitosis, and then cell lines of different (but diploid) genetic constitution being produced—can be the basis of a mosaicism for UPD. This can be UPDmat/biparental or UPDpat/biparental mosaicism, and it may be confined to the placenta or involve the fetus as well.

Complete UPD*pat*/normal mosaicism in the placenta (androgenetic/biparental mosaicism) leads to the histological phenotype of mesenchymal dysplasia (discussed in more detail on p. 396). If the UPDpat line also involves the fetus, features of Beckwith-Wiedemann syndrome may be present, and according to the cellular distribution of the UPD tissue, a more complex clinical picture may be observed (Wilson et al., 2008; Jalil et al., 2009).

Two unique cases cast light on how aberrant chromosomal behavior in the perizygotic period can lead to UPD-related pathology. A 46,XX/46,XY male child described in Strain et al. (1995) with growth asymmetry had complete maternal isodisomy in the 46,XX cell line and biparental inheritance in the 46,XY line. It may be that an ovum had completed a mitosis on its own, and then one of its daughter cells received the sperm (for the 46,XY line) while the other underwent endoreduplication (for the 46,XX,upd(mat) line): thus, biparental/gynogenetic mosaicism. A diploid sperm may have been the basis of the case in Hsu et al. (2008), in which the pregnancy from an apparently normal IVF embryo ended in intrauterine fetal death, with a portion of the placenta of molar appearance, at 14 weeks. Three separate genetic constitutions could be determined. The molar tissue was androgenetic 46,XX,uphd(pat); the fetus and placenta, both 46,XX, shared the same maternal genome but had different paternal genomes. Any explanation for this circumstance is necessarily complex.

Genetic Counseling

Uniparental Disomy for Individual Chromosomes

No instance of recurrence of full UPD for a particular chromosome, with a 46,XX or 46,XY karyotype, is known, and we assume there to be no discernibly increased recurrence risk. The association with increasing childbearing age is to be noted, but in reality the increase in risk for older mothers would be very small.

Segmental Uniparental Disomy

Segmental UPD arising postzygotically, and which is karyotypically 46,XX or 46,XY, we presume to imply no increased risk. UPD due to rearrangement would have a risk according to the nature of the specific rearrangement.

Four “Imprinting Syndromes” with More Than One Genetic Basis

Beckwith-Wiedemann Syndrome

The considerable majority (about 85%) of Beckwith-Wiedemann syndrome (BWS) occurs sporadically, including the two more common categories of UPD 11 and epigenetic error. The other categories that may have an important recurrence risk are recognized either by an abnormal cytogenetic report and/or by a positive family history. A detailed treatment is given in Weksberg et al. (2010).

Uniparental Disomy 11.

About a fifth of sporadic cases are due to mosaic segmental paternal UPD of 11p. This category of BWS can be suspected clinically if there is hemihyperplasia. No increased recurrence risk applies in the setting of segmental UPD and a normal karyotype.

Epigenetic Error.

In sporadic BWS with biparental disomy, the underlying cause is an epigenetic error (“epimutation”) affecting the ovum or early conceptus. This is the basis of a little over half of all cases. There is biparental inheritance with aberrant methylation on the maternal chromosome of either DMR1 (gain of methylation, ~5% of cases) or DMR2 (loss of methylation, ~50% of cases), the latter combination particularly associated with IVF. No cases of recurrence in this setting are known, and this fits the understanding of a typical postzygotic generation of the epimutation (Scott et al., 2008b) (but note recurrences in the section on “Epigenetic Silver-Russell Syndrome”). Theoretically, there might be a very small increased risk, if the same susceptibility factors (subfertility, IVF) were operating.

11p Rearrangement.

Chromosome rearrangements are rare causes of BWS. A *balanced* reciprocal translocation or an inversion with one breakpoint in distal 11p, if of maternal transmission, may lead to BWS (Li et al., 1998). An *unbalanced* distal 11p15 duplication, if of paternal origin, leads to double expression of *IGF2* in the 11p15 region, and this brings about the growth pattern of BWS (and if of maternal origin, a Silver-Russell growth retardation phenotype results; see later). Functional trisomy of nonimprinted 11p segments, or other imbalance due to a translocation, may contribute to the clinical picture (Han et al., 2006; Russo et al., 2006; South et al., 2008c; Bliek et al., 2009). The recurrence risks for these various circumstances will depend upon the nature of the rearrangement, and the parental karyotypes.

Mendelian Mutation.

Autosomal dominant BWS accounts for about 10% of cases, the major locus *CDKN1C*. Typically, only the offspring of female heterozygotes are affected. Careful review of the pedigree in the maternal line is necessary to identify mildly affected individuals, and bearing in mind the amelioration of phenotype with time (Hunter and Allanson, 1994; Elliott et al., 1994). One might consider the very rare case of deletion of the differentially methylated region DMR1 also to be in the category of Mendelian mutation; a child receiving this deletion from a mother has BWS, due to consequential biallelic *IGF2* expression (Sparago et al., 2004). A rare recessive basis of a maternal susceptibility to have a BWS child resides in the *NLRP2* gene, with failure to impose a proper imprint upon ova (Meyer et al., 2009).

Silver-Russell Syndrome

Most SRS can be traced to an anomaly of chromosome 7 (UPD) or chromosome 11p15 (epimutation), the latter somewhat mirroring the mechanism in BWS, as outlined earlier. Both these genetic forms typically imply a low risk of recurrence (Eggermann et al., 2010a).

Chromosome 7.

Upd(7)mat is seen in up to 10% of cases, and the clinical phenotype in this group is typically more severe (Binder et al., 2008). Sporadic occurrence has been the universal observation.

Chromosome 11.

Epimutations of 11p15, with hypomethylation of the *IGF2/H19* differentially methylated region (DMR1), comprise the largest single category: according to the stringency of clinical criteria, these account for about half of all SRS. This category may be associated with conception by IVF (Wakeling et al., 2010). Sporadic occurrence is very much the rule, albeit that very rare recurrences are on record, due to parental heterozygosity or parental gonadal mosaicism (Bartholdi et al., 2009). Structural rearrangement of 11p15, such as microduplication involving the differentially methylated region DMR2, is a rare cause (Eggermann et al., 2009). A familial translocation, such as the t(11;15)(p15.5;p12) described in Eggermann et al. (2010c), in which one segment comprises distal 11p, could lead to either SRS, if maternally transmitted, or BWS, if from the father (this is analogous to the scenario noted later for 15q11q13 rearrangements, with different phenotypes according to the parent of transmission).

SRS Phenocopies.

A SRS-like clinical picture can result from genomic changes elsewhere on the karyotype (Bruce et al., 2010).

Prader-Willi Syndrome

A summary of the different genetic forms of PWS, and the associated risks of recurrence, is set out in Tables 22–2 and 22–3.

Table 22–3. Approximate Relative Frequencies and Recurrence Risks, to Parents Having Had an Affected Child, for the Different Categories of Prader-Willi and Angelman Syndromes

	CATEGORY	RELATIVE FREQUENCY	RECURRENCE RISK
Prader-Willi syndrome	Classical deletion	70%	Extremely low*
	upd(15)mat	25%	Extremely low*
	Imprinting center defect	2%–5%	50%
	15q translocation/inversion	Rare	According to rearrangement
Angelman syndrome	Classical deletion	70%	Extremely low**
	upd(15)pat	3%–5%	Extremely low*
	Imprinting center defect	2%–5%	50%
	UBE3A mutation	10%–15%	50%
	15q translocation/inversion	Rare	According to rearrangement
	Epigenetic error/unknown	10%	Presumed very low

* No case yet recorded.

** Only one case in the world recorded (Kokkonen and Leisti, 2000).

Classical Deletion 15q11q13.

The empiric observation of zero recurrences out of some thousands of “trials” underscores the considerable unlikelihood of significant paternal gonadal mosaicism for the deletion observed in the PWS child. This is the basis of the substantial optimism that can be offered to parents in terms of any further pregnancies. A figure of around 0.1% may be a fair one to offer for the risk of recurrence. Nevertheless, the theoretical possibility of paternal gonadal mosaicism, or of a paternal predisposition to undergo chromosome 15 deletion in spermatogenesis (Molina et al., 2010), obliges acknowledgment that the risk is not zero. If prenatal diagnosis is pursued, CVS can be offered using FISH for deletions, or the *SNRPN* methylation test (Buiting et al., 1998).

Uniparental Disomy 15, Karyotype 46,XX or 46,XY.

We know of no recorded instance of recurrence of upd(15)mat PWS in a chromosomally normal couple, and we would otherwise assume, on theoretical grounds, any increased risk in a future pregnancy to be practically negligible, the modest maternal age effect notwithstanding.

Functional Defect (“Epimutation”) of Prader-Willi Syndrome Imprinting Center.

These extremely rare cases of IC defects will require individual expert advice. They can be suspected if a child has typical PWS clinically, but there is neither classical deletion nor UPD demonstrable. All cases of functional IC deficiency have so far been sporadic (but very few are known).

Prader-Willi Syndrome Imprinting Center Microdeletion.

The recognition of these cases will require referral to a specialist laboratory. A positive family history, if observed, would oblige the assumption of this category, unless or until otherwise proven. Assuming the father carries the genetic defect, the recurrence risk is high, namely 50%. *SNRPN* methylation testing on CVS can identify an affected pregnancy. The father's brothers would have a 50% likelihood to be heterozygous (making the assumption that their mother would carry the mutation), and in that case, these brothers would also have a 50% risk to have a PWS child. Equally, his sisters could be carriers, but their children would all be unaffected, and it would only be *their* sons who might, in the next generation, have the risk for a PWS child. The siblings of the affected child would themselves have no different genetic risk than the general population. The reader should work through the reasoning behind these various risk assessments, even though most counselors will never encounter this actual circumstance in the clinic.

Uncommon Cytogenetically Detectable Rearrangement.

The nature of the rearrangement (see the section on “Biology”), and the parental karyotypes, will determine the recurrence risk in each type.

PWS Phenocopies.

A duplication of the segment Xq21.1–q21.31 may lead to a clinical picture very reminiscent of PWS in the older child, while deletions distal to Xq25 are associated with a phenotype more resembling infantile PWS (Gabbett et al., 2008). A small fraction of males with fragile X syndrome present a PWS-like phenotype (p. 246). A focused Xq analysis, and a fragile X study, may thus be warranted in patients in whom a diagnosis of PWS is suspected on clinical grounds, but in whom chromosome 15 tests are normal. Deletion at 6q16.2, and the 1p36 deletion, may also be associated with a PWS-like clinical picture (D’Angelo et al., 2006; Varela et al., 2006; Bonaglia et al., 2008).

Angelman Syndrome

A summary of the different genetic forms of AS, and the associated risks of recurrence, is set out in Table 22–2. More detail is available in the reviews of Stalker and Williams (1998), Clayton-Smith and Laan (2003), and Van Buggenhout and Fryns (2009).

The clinical diagnosis of AS is sometimes easy (parents have recognized the condition in their child having seen a television program), but at other times, more challenging. Of course if accurate genetic advice is to be given, an accurate clinical diagnosis is crucial. The possibility of Rett syndrome may need to be considered (Scheffer et al., 1990).

A proposed very simple clinical screening test is to gauge the response to a vibrating tuning fork held close to one ear: an AS child will laugh and turn toward it (Hall and Cadle, 2002). The counselor must take the trouble to obtain a detailed family history. A genetic defect could have been transmitted through males for some generations, and only causing AS when it had been passed from a daughter of such a male. Figure **22–6** shows a family in which some quite distant relatives, including second cousins once removed and first cousins twice removed, had AS due to an inherited *UBE3A* mutation.

Classical Deletion 15q11q13.

Similarly to PWS, about 70% of AS is due to a de novo interstitial deletion. Only one case in the world is recorded of recurrence in siblings of a typically sized deletion. This case involved, presumably, a mother with gonadal mosaicism (Kokkonen and Leisti, 2000). Thus, as for classical deletion-PWS, we presume a very low—but clearly not zero—recurrence risk. There are two recorded examples of deletion AS in cousins, which manifestly represented coincidental de novo events in these families, in that different ancestral chromosomes were involved (Connerton-Moyer et al., 1997). The comments on prenatal diagnosis in PWS (see earlier) apply similarly here.

Uniparental Disomy 15, Karyotype 46,XX or 46,XY, Parents' Chromosomes Normal.

AS due to paternal UPD 15 is rare; as discussed earlier, the initial error may actually reflect a maternal age effect. Interestingly, the AS phenotype may be somewhat milder in UPD15, and in some children it was only after an electroencephalogram (EEG) showed typical findings that the diagnosis was suspected (Bottani et al., 1994). But this does not mean that some upd(15)pat AS children may not be severely affected (Prasad and Wagstaff, 1997). No recurrence is on record (Chan et al., 1993), and we assume on theoretical grounds that no usefully measurable increased risk would exist.

Angelman Syndrome Imprinting Center Microdeletion.

Assuming the mother carries the genetic defect, there is a high recurrence risk, namely, 50%. *SNRPN* methylation testing on CVS can identify an affected pregnancy. The possibility of maternal gonadal mosaicism for an IC mutation complicates the picture (Stalker et al., 1998). As noted earlier, but vice versa for AS, the siblings of the carrier mother could also be carriers (assuming their father to be heterozygous). However, it would only be the sisters who would have the risk for an AS child.

Functional Defect ("Epimutation") of Angelman Syndrome Imprinting Center.

The previous comments on PWS apply similarly here. All cases of AS due to a functional IC defect have so far been sporadic, but it would be prudent to offer prenatal diagnosis in a subsequent pregnancy (*SNRPN* methylation testing). Although the numbers are very small (but epimutation-AS is rare), there are grounds for supposing there might be a link with infertility/IVF (Manipalviratn et al., 2009). Had there been such a reproductive history, this fact would need to be weighed.

UBE3A Mutation.

If the mother carries the mutation, the risk for recurrence is 50%. Maternal mosaicism has been recognized (Malzac et al., 1998; Hosoki et al., 2005), and so nondemonstration of the mutation in the mother does not necessarily exclude a genetic risk. Indeed, it may be that such mosaicism is not uncommon (Stalker et al., 1998). It may be appropriate to track the mutation through the patrilineal family, in order to be able to offer genetic counseling to female cousins who might be carriers. The reader should study the illustrative pedigree in Figure **22–6**. There is the practical point that routine clinical testing for *UBE3A* is available in only a very few laboratories, and at some cost.

No Genetic Defect Demonstrable.

In a small fraction of AS, about 10%, no cytogenetic or molecular defect, nor *UBE3A* mutation, is demonstrable. Some of these cases could conceivably reflect a mutation that has not been able to be detected. The family history, if positive, may compel the assumption of a mutation, and thus imply a high recurrence risk. A negative family history might support the inference of a low risk, but it would not allow a definite assumption. If a normal sibling carried the same 15q11q13 haplotype, using DNA markers, a low-risk scenario would be probable. Expert advice should be sought.

Uncommon Cytogenetically Detectable Rearrangement.

The nature of the rearrangement (see the "Biology" section), and the parental karyotypes, will determine the recurrence risk in each type. The rare circumstance of UPD associated with a parental Robertsonian translocation is noted on p. 152.

Angelman Syndrome Phenocopies.

Rett syndrome has considerable clinical overlap with AS, as do the 22q13.3 deletion and Pitt-Hopkins syndrome (Hitchins et al., 2004; Takano et al., 2010).

A Simplification for Angelman Syndrome

Some parents will not find it easy to come to grips with these various possible causes for their child's condition even if, in the end, they need only consider the category that applies to themselves. It may be helpful to discuss AS, and the risks of recurrence, in the following terms. Let us say that AS is due, simply, to a lack of the UBE3A protein, a very important protein that is necessary for the brain to grow normally. The gene for *UBE3A* works only on the chromosome 15 from the mother, while the gene on the father's chromosome is dormant. There is a switch on the mother's chromosome that makes this gene work.

- If the bit of the maternal chromosome that contains this gene is missing (deletion), or if the mother's chromosome is replaced by another one from the father (UPD), no UBE3A protein can be made. These two types happen as one-off events.
- If the switch fails on the mother's chromosome, then the gene remains dormant, and no protein is made (imprinting center fault). This type can happen one-off, as though the switch "gets stuck," for reasons that we do not well understand. Or there may be a genetic fault in the actual switch, and in this case the defect could be passed to a subsequent child.
- If the *UBE3A* gene itself is faulty on the mother's chromosome (mutation), no protein is made, or only an abnormal protein that cannot function. The genetic risk depends on whether the faulty gene started with the child (no increased risk) or if the mother is a carrier (high risk). Note that the mother can be a carrier and still be perfectly normal, since the faulty gene would be the one she got from her father, and so in any event it would be switched off.
- Sometimes the *UBE3A* gene fails to work, even though the maternal chromosome is normal and has a normal switch. We do not know why this happens (there has been a suggestion that one cause *may* be if there had been difficulty achieving the pregnancy, either naturally or with IVF). This type is a one-off event.

A common question parents have is whether their normal children might, in the next generation, have an AS child. Or the aunts and uncles of an AS child might want advice about risks to their future children or to their grandchildren. The answers are as follows.

- The normal siblings of an AS child have no increased risk, for any genetic category, with the possible exception of a familial translocation. Even if the AS child has (or had) a potentially heritable type of *UBE3A* genetic defect, the fact that the sibs themselves are normal declares that they cannot have received it. If they had got the abnormal gene, they would have AS; since they do not have AS, they cannot have the gene. The sex of the siblings is immaterial.
- Aunts and uncles have an increased risk for children or grandchildren of theirs only if a heritable type of AS is involved (imprinting center defect, *UBE3A* mutation). In that case, an uncle could be a carrier, but his children would not be at risk, since the *UBE3A* gene would be dormant anyway. Daughters of his, however, could have an AS child. A carrier aunt would have a high risk (50%) to have an AS child. But her grandchildren, through her normal sons and daughters, would have no increased risk. Her

normal children would have declared themselves, by their very normality, not to have inherited the genetic defect.

Prenatal Testing Considerations

The American College of Medical Genetics has recommended testing for UPD be offered when an imprintable chromosome (6, 7, 11, 14, 15) has raised a concern at prenatal diagnosis, either with level II mosaicism, or especially in the context of a Robertsonian translocation, or if there are indicative ultrasound findings (Shaffer et al., 2001). We note that this advice is somewhat more liberal than that of Kotzot (2008b), who has suggested that only chromosome 14 and 15 be considered in this regard (and see p. [link]).

Uniparental Disomy for the Entire Chromosomal Complement

UPD for the entire *paternal* chromosome set (hydatidiform mole) is associated with an increased recurrence risk; this is discussed in detail in Chapter 23 (p. 400). There is no discernibly increased risk for the recurrence of UPD for the entire *maternal* chromosome set (ovarian teratoma).

Notes:

1 As a general rule, the abbreviation is in uppercase (UPD, UPHD, UPID) when making broad reference to the concept of uniparental disomy, and in lowercase (upd, uphd, upid), according to the rules of cytogenetic nomenclature, when attention is more focused upon a specific case.

2 The subject is, however, becoming more complicated, with the recognition that some nonimprintable genes may normally function monoallelically, and that different parent-of-origin epigenetic influences upon many loci can operate in different anatomical brain regions during development (Gimelbrant et al., 2007; Gregg et al., 2010).

3 It might be more accurate to speak of a “failed rescue,” or better a “foiled rescue,” since the end result is an unfortunate one. Or “mistaken correction.”

9 The same mechanism of postzygotic recombination may suggest itself in the setting of somatic mosaicism for a mendelian condition. Happle and König (1999) discuss the case of a boy with a rare skin condition (epidermolytic hyperkeratosis of Brocq) affecting most of his body, but with some areas of more severely affected skin, and some areas which were healthy. Imagining that the Brocq locus might be in the distal short arm of the chromosomes depicted in Figure 20–3 and with the black chromosome having the mutant allele, the typically affected skin would have the genotype represented in (*a*), the more severely affected skin would be in (*f*), and the normal skin in (*e*).

4 There is multiple nomenclature of these regions. DMR1 and DMR2 may be referred to as Imprinting Control Regions 1 and 2, ICR1 and ICR2. DMR1 is also known as H19 DMR, and the telomeric cluster; and DMR2 as KvDMR1, KCNQ1OT1 DMR, LIT1 DMR, and the centromeric cluster.

5 In BWS with isolated DMR2 loss of methylation (the most common form; see Table 22–1), Wilms tumor is not seen, and this knowledge can inform clinical management.

6 An aide-mémoire: Prader-Willi due to Paternal deletion.

7 An additional copy of this gene leads to hyperpigmentation (Akahoshi et al., 2001). This is a nice example of a dosage effect: one copy of the *P* gene = pale skin, two copies = normal pigmentation, three copies = hyperpigmentation.

8 The word *mutation* is normally taken to indicate that there is a change in the DNA sequence (from the Latin *mutare*, to change). By definition, no such change has occurred in an epimutation. But there has been a change in the functioning of the DNA.







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Chromosome Abnormalities and Genetic Counseling (4 ed.)

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Gametogenesis and Conception, Pregnancy Loss and Infertility

Chapter: Gametogenesis and Conception, Pregnancy Loss and Infertility
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HUMAN CONCEPTION and pregnancy is both a vulnerable and a robust process. Vulnerable, in that a large proportion of all conceptions are chromosomally abnormal, with the great majority of such pregnancies aborting. Robust, in that more than 99% of the time, a term pregnancy results in a chromosomally normal baby. Unbalanced chromosomal abnormalities are seen in less than 1% of newborns (see Table 1–3 in Chapter 1). But the economic cost of chromosomally abnormal conceptions is not horrendous; it is measured largely in terms of miscarriage, seen or unseen. The occasional chromosomally abnormal child is, relatively speaking, an exceptional outcome—the tip of an iceberg (Fig. **23–1**).

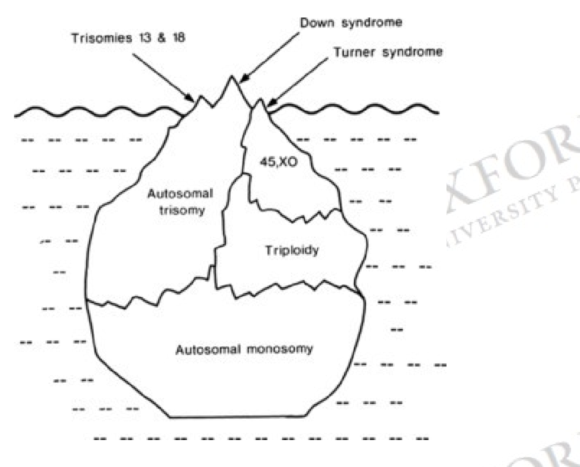




Figure 23–1
The iceberg of chromosomal pregnancy loss.

Most of this chromosomal vulnerability lies in the process of producing eggs and sperm. Meiosis hangs, literally and figuratively, upon "tender filaments," and often the meiotic chromosomes are incorrectly distributed to the daughter cells. Indeed, humans are more prone to produce aneuploid germ cells than any other species studied (McFadden and Friedman, 1997).[1] The group who are particularly likely to produce abnormal gametes are carriers of balanced chromosome rearrangements, and much of this book is devoted to that fact.

Advances in reproductive technology now enable many otherwise infertile couples to have children. Translocation carriers may have recourse to preimplantation genetic diagnosis (PGD) as a means to improve their chances of achieving a successful pregnancy (Chapter 26). In the case of men with poor sperm production, intracytoplasmic sperm injection (ICSI) at in vitro fertilization (IVF) is a means to get a single sperm into an egg. Success with IVF is not necessarily easy to achieve, neither is it a certain outcome, and counselors dealing with infertile couples need a particular awareness of the psychological and practical difficulties they may face (Boivin et al., 2001). A "failed embryo transfer" following IVF may be considered as a form of pregnancy loss not unlike that of the natural miscarriage of a wanted pregnancy.

Biology

Gametic Cytogenetics

Many more sperm are made than eggs, by orders of magnitude, and logically one might have expected a higher standard of meiotic fidelity in the scarcer gamete (Hunt and Hassold, 2002). But in fact it is the other way round, and so it is the egg that commands most of our attention in terms of the practical relevance of gametic chromosomal pathology.

Oöcytes and polar bodies

IVF is widely applied in the management of infertility, and one consequential benefit of this has been the access afforded to study of the oöcyte and its minor partner, the polar body. Many eggs sampled prove to be surplus to the requirements of the couple, and they are often willingly donated for research. Pellestor et al. (2006) have reviewed the literature, in which different groups applied various methodologies; and Fragouli et al. (2006b) report a four-city (Dundee, London, Rochester, and New Haven) collaboration, in which comparative genomic hybridization (CGH) was applied, with the view that this technique would be less prone to artifactual misinterpretation. Overall, a fifth to a quarter of oöcytes are cytogenetically abnormal. In the CGH study, many eggs and polar bodies could be analyzed as a pair, allowing the detection of reciprocal imbalances to function as an internal control. Most of the abnormalities are accounted for by hyperhaploidy with a 24-chromosome count (an additional double-chromatid or single-chromatid chromosome), hypohaploidy with a 22-chromosome count (a missing homolog) or a 23-chromosome count that could be described as “22½” (one homolog represented by only a single-chromatid chromosome), and diploidy with 46 chromosomes.

In order to address what might be the basis for these abnormalities, Cupisti et al. (2003) analyzed a set of eight chromosomes (large, medium, and small representatives), from pairs of oöcytes and their first polar bodies. (These were spare eggs, 236 in all, from women presenting for infertility treatment due to a variety of causes, both male- and female-based.) The large chromosome (1 being the chosen representative) had not misdivided, and only the X of the three medium-sized chromosomes (9, 12, X) had not. Of the smaller chromosomes (13, 16, 18, 21), all were seen in aneuploid state, and for each chromosome, 2%–3% of eggs displayed aneuploidy. As these authors note, this rate cannot be applied to all chromosomes (23 × 2%–3% would come to a figure of 46%–69% as the rate for all chromosomes combined, certainly an overestimate); the smaller chromosomes apparently are more prone to erroneous behavior. Different mechanisms could be discerned: classical nondisjunction, predivision (see p. [link]), and gonadal mosaicism. The aneuploidy rates correlate with increasing maternal age, most notably in the single-chromatid nondisjunction category (Pellestor et al., 2003).

All these data are necessarily influenced by the source of the material: the ova mostly come from women being treated for infertility, who are typically of an older childbearing age, and some of the ova may be of poor quality, although it is of interest that the distribution of abnormality does not differ according to the infertility being due to the female or to the male (Pellestor et al., 2002). What does seem clear is that in the vicinity of 20%–25% of eggs from this population of women are chromosomally abnormal, about half of these manifesting as full aneuploidy. Maternal age is a most important correlate, and this link is well illustrated in the work of Battaglia et al. (1996; and see Fig. 3–7 in Chapter 3), showing how the structural integrity of the oöcyte's meiotic apparatus declines as a woman gets older.

One particular type of abnormal egg, the giant binucleate oöcyte, is typically diploid (Balakier et al., 2002; Rosenbusch et al., 2002).

Sperm

The gamete whose chromosomes are most readily accessible to analysis is the sperm. The earlier sperm karyotyping studies used the “humster” (human sperm + hamster ovum pseudofertilization) test. Guttenbach et al. (1997) reviewed the findings from eight research groups in the field, combining a total of over 20,000 sperm karyotypes from healthy donor men using the humster test. From this accumulated experience, the conclusion was that around 10% of sperm are chromosomally abnormal. Aneuploidy is observed in 1%–3%, and about another 5%–10% of sperm have structural chromosome abnormalities, many of which were presumed to have arisen during spermiogenesis as an immediate postmeiotic event.

Fluorescence in situ hybridization (FISH) analysis has enabled chromosome counts to be made on very large numbers of sperm. This approach bypassed any question that the humster test might have selected against abnormal sperm, although in fact it appears that this did not happen (Van Hummelen et al., 1996). Shi and Martin (2000b) reviewed the published experience, observing that over 5,000,000 sperm from about 500 normal men have been analyzed in a number of laboratories around the world, using one-, two-, or three-probe FISH. Considerable variation existed between subjects, probably a biological effect; interlaboratory variation was also noted, presumably reflecting local methodological differences. The average disomy rate for each of the autosomes ranges around the 0.1%–0.2% mark. The figure for chromosome 14 being a little above, at 0.4%, may be artifactual, while higher levels in the G group chromosomes, nos. 21 and 22, are more likely to reflect reality. X+Y disomy is observed in 0.25%. No ethnic differences have come to light, at any rate comparing Caucasians and Chinese (Shi and Martin, 2000a). Neither is there any, or at least any consistently observed, correlation with paternal age, except possibly with respect to XY disomy.

An increased rate of sperm aneuploidy may influence the success of conception, at least as measured at IVF and PGD. A higher rate of sperm abnormality is associated with a lesser rate of success following ICSI, and with a higher rate of aneuploidy in preimplantation embryos (Nicopoullos et al., 2008; Rubio et al., 2009); and consistent with these observations, the outlook for men with increased aneuploidy is improved by preimplantation diagnosis for aneuploidy screening (PGD-AS) (Rodrigo et al., 2010) (see also later section on “Recurrent Implantation Failure”).

Fathers of Aneuploid Children.

There are very few data on 46,XY men who have actually fathered children with chromosomal abnormalities. Hixon et al. (1998) studied 10 fathers of paternal-error Down syndrome children (the error at meiosis I in three, meiosis II in six, postzygotic in one) and found no differences: these fathers had a mean of 0.15% disomic 21 sperm versus 0.17% in the controls. Similarly, Blanco et al. (1998) studied a group of 15 fathers of children with trisomy 21, and, overall, the fraction of disomic 21 sperm was little different from a control group: 0.31% versus 0.37%. However, of the total of 25 fathers from the two studies, three stood out with twice the level of sperm disomy 21; and two of these also showed an increase in sperm disomic for chromosomes 13 and 22 (Soares et al., 2001a). As for monosomy X Turner syndrome, three-quarters of which may be the consequence of nullisomy X in the sperm, four fathers of TS daughters reported in Martínez-Pasarell et al. (1999) and in Soares et al. (2001b) had increased levels of sperm with a sex chromosome aneuploidy, and also with disomies 13, 21, and 22. From the foregoing, it is tempting to suppose that a minority of normally fertile men may be predisposed to meiotic errors at spermatogenesis, whether generalized or restricted to one chromosome; but the data are as yet too insubstantial to make a firm statement.

Whether meiotic recombination occurs may be an important factor influencing the integrity of the disjunctional process (a point well established in female meiosis). Savage et al. (1998) demonstrated that among paternally derived trisomy 21 cases, the rate of chromosome 21 recombination was only about half the expected level. Likewise, Thomas and Hassold (2003) discuss that recombination is very considerably reduced in the pseudoautosomal region (PAR1) of males with 47,XXY Klinefelter syndrome, in whom the causative meiotic error had been shown to be paternal. The conceptions in these cases had been, presumably, 24,XY sperm + 23,X ovum. Shi et al. (2001b) provide corroborative support for this concept in their analysis of sperm from a normal male, this man being usefully heterozygous for two markers in the pseudoautosomal region (*DXYS15* and *STS*). As does any normal man, he produced some disomic 24,XY sperm, among the normal 23,X and 23,Y sperm. Comparing the normal and the disomic sperm in this subject, recombination occurred between the two markers in 38% of the former, and in only 25% of the latter.

Men With a Chromosomal Abnormality.

Sperm karyotyping studies in men who themselves have an abnormal karyotype, whether balanced (e.g., a reciprocal translocation) or unbalanced (e.g., 47,XYY), are dealt with in the chapters on the particular abnormality. A rather different type of study of the chromosomes—an assessment of their spatial organization within the nucleus—has been applied to translocation carriers; and compared with normal controls, subtle differences in the “packaging” of the chromosomes can be discerned (Wiland et al., 2008) (see also later section on “Factors in the Male”).

Cytogenetics of the Very Early Conceptus

An aneuploid gamete (nullisomic or disomic) will lead to an aneuploid conceptus (monosomic or trisomic). A diploid gamete, combining with a normal gamete, will give rise to a triploid conceptus. On simple arithmetic, given that around 20% of oöcytes may be aneuploid and 10% of sperm are abnormal, and simplistically supposing equal fertilizing/fertilizable capacity, the expectation is for about 30% of conceptions to be chromosomally unbalanced. On top of this, dispermy (two sperm fertilizing the one ovum) can cause triploidy. An abnormal postzygotic cell division can give rise to mosaicism, and this may be a common happening (discussed later). A very rare event is the

generation of uniparental disomy due to two gametes being coincidentally nullisomic and disomic for the same chromosome. These several possibilities all add up to a substantial potential for chromosome abnormality in the very early conceptus, in the first days of existence.

The cleavage embryo ("pre-embryo") (days 1–3)

The development of the technology of PGD in association with IVF has offered a much clearer view of the frequency of chromosomal abnormalities in the zygote and in the first few days post conception.[2] Admittedly, couples presenting for PGD will not, in the main, be a true representation of all couples. One category of patient will, however, be close to "chromosomally typical": otherwise normally fertile women who are heterozygotes for a mendelian condition, presenting for diagnosis of embryonic sex or for specific mutation testing. These embryos offer the best insight to the true background rate of chromosomal abnormality, with respect to the maternal age groups involved. Pellicer et al. (1999), assessing some or all of chromosomes 13, 16, 18, 21, 22, X and Y, studied 10 mendelian heterozygous women of mean age 34 years, range 30–36. These women had a total of 12 abnormal embryos out of 62 tested (19%), but a considerably higher figure (46%) was observed in a group of older mothers presenting for the same reason (see also Table 23–3).

Table 23–3. Chromosome Abnormalities in a Series of Candidate Couples for Intracytoplasmic Sperm Injection (ICSI), Comprising 2196 Men and 1012 Women (Some Female Partners Not Studied, Since It Had Been Assumed the Infertility Was in the Male)

KARYOTYPE	NO. (FEMALE)	NO. (MALE)
*Sex chromosomal**		
45,X/46,XY**	–	8
47,XXY	–	49
47,XXY/46,XY, etc.***	–	8
47,XYY****	–	8
Structural Y abn.	–	9
X or Y rcp with autosome	–	5
Autosomal		
Simple rcp	7	18
Mosaic rcp	–	2
Complex rcp	–	2
rob	7	18
inv (pericentric)	7	3
Structural abn., unbalanced	–	4
Total abnormal	21 (2.1%)	134 (6.1%)
None of the above	991	2062
Totals	1012	2196

Note: Most, but not necessarily all, of these abnormalities will have been related causally to the infertility.

* Excluding a variety of low-level X chromosome mosaicisms in 28 women, of doubtful significance (including them would increase the "Total abnormal" fraction to 4.9%).

** Including one 45,X/46,XX/46,XY low-level mosaic.

*** Including one 47,XXY/48,XXXY/46,XY mosaic and one low-level 47,XXX/47,XXY/46,XY mosaic.

**** Including one 47,XYY/46,XY low-level mosaic.

Source: From a France-wide study 1995–1998 (Gekas et al., 2001).

The "atypical" group of patients presenting to the IVF clinic are of course a population of clinical interest, and thus the observations gained from study of them, however unrepresentative they might possibly be of the general population, are very germane to the agenda of the counselor. Numerous studies confirm that a high fraction of unused IVF embryos are karyotypically abnormal, the ranges observed from 30% to 65% (Wilton, 2002). Phenotypically abnormal embryos with multinucleate blastomeres are very likely to be chromosomally abnormal (Kligman et al., 1996). Bahçe et al. (1999), in a study of day 3 embryos, looked at aneuploidies not typically associated with clinical miscarriage or with term pregnancy, and showed that trisomies 1, 15, and 17 occur not infrequently. Voullaire et al. (2000b) applied the technique of CGH to the study of embryos that had surpassed the 5-year statutory storage limit in the state of Victoria, Australia, comprising 12 embryos from eight women aged 26–33 years. Upon thawing and culturing, 3 of these 12 embryos were scored as of good morphology, 3 as average, and 6 as poor. Three out of three good-morphology embryos were chromosomally abnormal in one or more blastomeres, and likewise two out of three average-morphology embryos, and four out of six poor-morphology embryos: thus, only a quarter of embryos were normal. Many of the aneuploidies were in mosaic state.

Mosaicism of the very early embryo has been one of the more startling discoveries to emerge from the PGD laboratory, although again there is the caveat that it would be unsafe to draw too many conclusions from these laboratory observations concerning the risk for mosaicism in chromosomally normal zygotes, conceived naturally. Many IVF

embryos are aneuploid or diploid/haploid mosaics, and even in normal-appearing embryos the fraction, analyzing a limited number of chromosomes, ranges from 17% to 43% (Wilton, 2002). Iwarsson et al. (1999), applying FISH for chromosomes 15, 16, 17, 18, X and Y, found as many as 72% of “good-morphology” freeze-thawed embryos to be chromosomally abnormal, with 57% being diploid mosaics.

This mosaicism presumably reflects the fact that the first few postzygotic divisions are particularly susceptible to errors in chromosomal distribution. The main mechanism may be chromosome loss, due to anaphase lag, but chromosome gain is also frequent; mitotic nondisjunction (different cells with the corresponding monosomy and trisomy) occurs in a small minority (Daphnis et al., 2008). When the mosaicism is extensive (several different karyotypes), these embryos may be referred to as being chromosomally “chaotic.” As noted earlier, Voullaire et al. (2000b) showed, using CGH methodology to check on every chromosome, that a majority of surplus embryos of IVF patients were mosaic. A CGH study by Blennow et al. (2001), on embryos from translocation carriers which had been diagnosed as aneuploid on PGD, demonstrated in some that every cell could be different: the absolute maximum mosaicism.

The morula and blastocyst (days 4–5)

The short period during which the cleavage embryo advances through the morula stage and into the blastocyst may be an important hurdle during which the development of many chromosomally abnormal pre-embryos arrest, in particular those with extensive mosaicism (Bielanska et al., 2002a). In the largest study of its kind, Fragouli et al. (2008) studied 136 embryos donated for research, of good quality, and which they considered would not differ too greatly from the in vivo situation (although acknowledging that the embryos came from couples undergoing treatment for infertility, and with an average maternal age of 36). They cultured these embryos through to the blastocyst stage.

Overall, 39% of these blastocysts were aneuploid, with 35 trisomies and 46 monosomies identified. As expected, the rate was considerably higher in older (37 and over) than in younger women, 48% versus 16%. The simplest state was a blastocyst with a single aneuploidy, with slightly more monosomies (e.g., monosomy 15) than trisomies (e.g., trisomy 19); and the most complicated imbalance was monosomy for six chromosomes and trisomy for five. Only chromosomes 3, 4, 5, and 9 were not represented among the aneuploidies. The number of monosomic blastocysts was an interesting observation: it had previously been considered that the monosomic conceptus could very rarely advance beyond the morula (day 4). The aneuploidy rate was close to the rate expected from the combination of sperm and egg rates, but less than that seen in cleavage-stage embryos, leading these workers to conclude that the abnormalities in the blastocysts were of meiotic origin; and in agreement with Bielanska et al. (2002a) that “chaotically mosaic” cleavage-stage embryos, aneuploid in many or all of their cells due to mitotic errors, will almost all fail at the embryo-morula hurdle.

Along similar lines, Daphnis et al. (2008) looked at embryos that had undergone blastomere biopsy and CGH analysis at day 3, cultured until day 5, and then every cell analyzed. None showed a nonmosaic aneuploidy, and thus it could be inferred that the initial zygote had been normal, with all abnormalities having arisen thereafter. From 13 embryos normal at day 3, 10 had become mosaic at day 5, with 6 of these “chaotic.” Of those abnormal at day 3, all remained abnormal, and mostly more so, at day 5, with 70% chaotic mosaic. Trisomy or monosomy of chromosomes 16, 18, and 22 were the most often observed aneuploidies. Partial aneuploidies were also observed; these may be generated at certain fragile sites. The conclusion from this study was that embryos normal at day 3 may retain normality by day 5, but an abnormality at day 3 predicts a poor fate.

Mosaicism that arises over the timeframe of days 4–5, but which is not chaotic, and with the existence of a normal euploid lineage, augurs for a progressive normalization of the embryo that can then, in due course, go on to produce a euploid fetus, and a normal child (Fragouli et al., 2008). The abnormal cells fall by the wayside.

The very early “embryo proper” (the third to the sixth weeks)

The embryo proper, in the sense that the body plan is beginning to be laid out, takes form in the third week, and is barely 1½ mm long; by the end of the sixth week postconception (8 weeks by dates), it will be 1½ cm. This is a timeframe that is not easily studied. First, this appears to be a period during which the threshold for natural abortion is relatively high, and many abnormal embryos seem able to maintain existence (however imperfect that existence might be). Second, the practicalities of collecting intact embryos from very early spontaneous miscarriage, they being scarcely discernible among the products of conception otherwise (chorionic villi the main component), present obvious drawbacks. Nevertheless, Philipp et al. (2003) were able to study by endoscopy the anatomy of embryos in women prior to uterine evacuation for “missed abortion”; in some, growth had arrested, thus enabling a window upon an earlier developmental stage than a standard calculation of gestation-by-dates might have indicated. Some embryos (illustrated in their paper) showed no recognizable external structures (e.g., trisomy 16), while in some an outline embryonic form could be recognized (e.g., trisomies 4, 7). A French group have been able to look at an even earlier stage (and in which growth arrest might not yet have happened), in examining expelled products following very early elective abortion using the drug mifepristone (RU486), following which procedure there is little, if any disruption of the embryonic anatomy (Golzio et al., 2006). One severely malformed embryo (Fig. **23–2**), at 25 days postconception age, had failed to develop such crucial organs as the forebrain, the mesonephros (the kidney anlage), or the liver; and the pharyngeal arch system, a fundamental structural framework for the upper body, was absent. (Neither had limb buds developed; but this is normal at this stage.) The heart was grossly distorted. The chromosomal diagnosis was trisomy 8 (nonmosaic), due to a parental meiotic nondisjunction. It may be that, following this approach, other such embryonic karyotypic-phenotypic correlations will be able to be drawn.

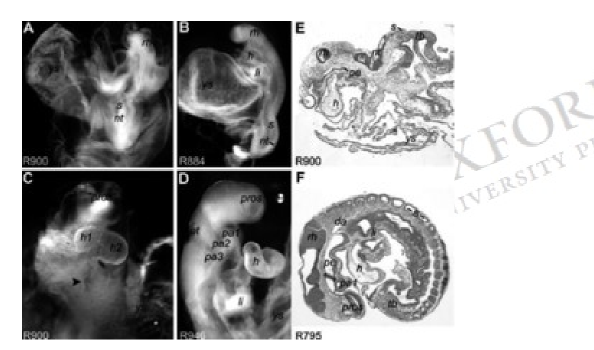




Figure 23–2
A very early embryo with nonmosaic trisomy 8 (*A*, *C*, and *E*), due to meiotic I nondisjunction. A normal embryo (*B*, *D*, and *F*) is shown for comparison; each is about 3 weeks post conception, and 3 mm in length. The trisomic embryo is devastatingly malformed. On sagittal section, normal brain structure (the prosencephalon [pros] and rhombencephalon [rh] nicely shown) and regular somite (s) development are clear to see in the normal embryo (F), compared with the gross deformity in the trisomic embryo (*E*). On ventral view of the trisomic embryo (*C*), the heart (h1, h2) is bifid, and no liver bud (li in the normal) has formed between it and the anterior intestinal portal (arrowhead). da, dorsal aorta; nt, neural tube; ot, otic vesicle; pa#, pharyngeal arch #; pe, pharyngeal endoderm; tb, tail bud; ys, yolk sac. (From C. Golzio et al., 2006, Cytogenetic and histological features of a human embryo with homogeneous chromosome 8 trisomy, *Prenatal Diagnosis* 26:1201–1205. Courtesy H. C. Etchevers; reproduced with the permission of Wiley-Blackwell.)

Cytogenetics of very Early Pregnancy Loss

Nonimplantation and occult abortion

Although the natural in vivo circumstance might differ from the observations in vitro, nevertheless it is a fair assumption that a substantial fraction of human conceptions have a lethal genetic burden and will not implant. It becomes a semantic question whether the existence of a nonimplanting morula or blastocyst could be described as a pregnancy, and whether its loss could be considered an abortion. Transient implantation may be associated with little or no perturbation of the menstrual cycle, although the woman may fleetingly feel pregnant as a hormonal response is briefly elicited. This is “occult abortion” (Miller et al., 1980). Monosomy, or extensive mosaicism, may be lethal even before the morula converts into the blastocyst, at least on in vitro observation, but some will reach the blastocyst stage, as discussed earlier. Some trisomies impart early lethality. Trisomy 1 may exist in a small fraction of day-3 embryos in an IVF population, and yet it is almost unknown in an established pregnancy (just four recorded cases, presenting as empty sac[3]; Vičić et al., 2008). Most trisomy 17 is apparently lost in similar circumstances, with a fall in its incidence from 12% at day 3 to only 0.2% in spontaneous abortions (Bahçe et al., 1999; Dunn et al., 2001). The frequency and range of aneuploidies seen in blastocysts is otherwise similar to that seen at the stage of late first-trimester miscarriage, and this is the next major period during which selective pressure is exerted (Cupisti et al., 2003).

Recurrent implantation failure at in vitro fertilization

More than one cause may apply, and a distinction is to be made between maternal (“uterine receptivity”) and embryo characteristics. A subgroup of couples undergoing IVF may produce apparently normal embryos, but suffer repeated implantation failure or recurrent pregnancy loss (RIF, RPL) following transfer of embryos to the uterus. Voullaire et al. (2007) compared the frequency of aneuploidy at PGD in embryos from woman with RIF and found a higher rate of complex chromosome abnormality, which they defined as aneuploidy of three or more chromosomes, compared with those who had not. This effect was not related to maternal age. Thus, it may be that, in at least some of these women, there is an underlying susceptibly for embryos of theirs to undergo chromosomal error during the first two rounds of mitosis.

Taking a step back, the gametes from RIF couples may be analyzed. Vialard et al. (2008) analyzed the first polar body of the oocyte and determined an average aneuploidy rate of 35% (range 0–86%). There may be a male factor: these workers also observed sperm aneuploidy rates of 2.1% in the male partner of RIF couples, compared with 0.6% from a comparison group of couples undergoing IVF, where a female factor had been identified as the cause of the infertility. Of 25 couples who had had both sperm and polar body studies, one-third demonstrated no increased chromosomal risk, but in 60%, there was an increased aneuploidy rate in either polar body or sperm, while in 8%, the gametes of both of the RIF partners displayed a chromosomal susceptibility. These observations would suggest that, in two-thirds of RIF couples, recurring meiotic error may be the cause.

Cytogenetics of Spontaneous Abortion and Later Pregnancy Loss

Clinical miscarriage

At the late first-trimester miscarriage, at which stage tissue from “products of conception” is more readily obtainable, we have a clearer idea of how many conceptuses are chromosomally abnormal and what the abnormalities are. Of all recognized pregnancies (recognized in the traditional way, that is), about 10%–15% end in clinical miscarriage—“spontaneous abortion”—mostly toward the end of the first trimester.[4] If the products of conception are successfully cultured and karyotyped, in most studies somewhat over a half of abortuses are shown to have a chromosome abnormality (a figure that has increased with increasing expertise and experience, and the application of modern methodologies: Menasha et al., 2005; Zhang et al., 2009c; Rajcan-Separovic et al., 2010). In the case of an empty sac, the fraction with a chromosomal abnormality may be slightly less, about 60%, compared with close to 70% in cases in which embryonic/fetal parts are identifiable on ultrasound (Lathi et al., 2007), and over 80% in those in which multiple external defects are observed through an endoscope prior to operative evacuation (Philipp et al., 2003).

Trisomies account for about 60% of all cytogenetic abnormalities identified at spontaneous abortion (Fig. **23–3**). The most commonly seen abnormal karyotypes are trisomy 16, monosomy X, and triploidy. Monosomy X and triploidy account for approximately 20% and 15% of all abnormalities, respectively; and as many as 1% of all human conceptions may have trisomy 16 (Benn, 1998). Double trisomy (trisomy for two chromosomes) is infrequent, this being an observation in 2.2% in one large series (Diego-Alvarez et al., 2006). After aneuploidies and triploidies, structural rearrangements constitute most of the remainder.

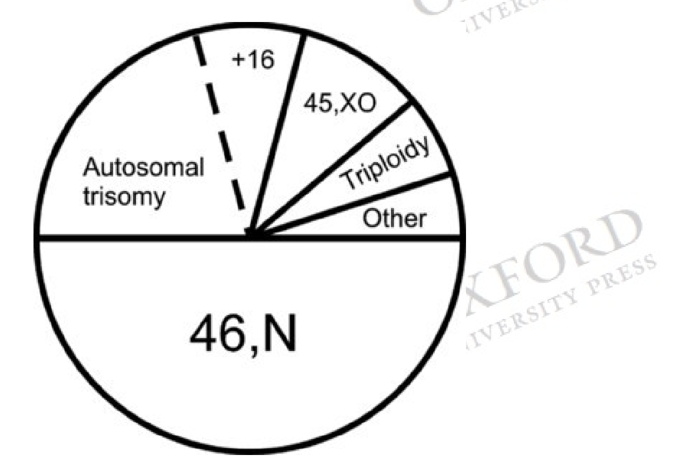


Figure 23–3

Chromosomal findings in products of conception from spontaneous abortion. (After Kajii et al., 1980.)

Molecular methodologies (MLPA and array-CGH) are powerful, but they may miss most triploidies. SNP arrays have a higher chance of detecting this chromosome state. This was conceptually demonstrated in a recent study based on CVS obtained from women at the time of diagnosis of a missed abortion (Morales et al., 2008). Karyotypes were obtained on 103, and 80% were abnormal. Because 12 were triploidies, the authors argue that semidirect analysis of CVS, which avoids long-term tissue culture, is a low-cost and effective tool to identify chromosome anomalies in miscarriages. The use of SNP arrays and genotyping can, in principle, detect these abnormal ploidy conditions. Zhang et al. (2009c) studied products of conception from 115 spontaneously miscarrying first-trimester pregnancies, applying standard cytogenetics, polymerase chain reaction (PCR)-based microsatellite genotyping, and array-CGH. As expected, cytogenetics in those for which culture was successful (80% of cases) revealed a substantial fraction (60%) with aneuploidy, including two with double trisomy. The molecular methodologies detected another 15 cases with abnormality, including some that had been normal on karyotyping, or which had failed to culture. The overall fraction with abnormality barely changed (61%), but the fraction of the whole material with an accurate genomic diagnosis was much increased. Likewise, Rajcan-Separovic et al. (2010) have tested the potential of array-CGH in revealing a greater fraction of imbalance in abnormal embryos with apparently normal karyotypes. FISH analysis of cells in celomic fluid from a “missed abortion” has been proposed as a means to ensure a more accurate cytogenetic diagnosis (Chatzimeletiou et al., 2005).

The origin of the abnormality is, in most, an error at maternal meiosis I, and this includes most of the major trisomies: trisomies 13, 14, 16, 21, and 22, with trisomy 18 a possible exception. Robinson et al. (1999) analyzed the originating status of certain of the less studied karyotypes: trisomies for chromosomes 2, 4 through 10, 12, 15, 17, and 20. About three-quarters showed three alleles for the trisomic chromosome, thus confirming a meiotic origin. Most of the remainder are presumed to have been due to somatic

errors; some might have been mosaic, but not detected as such. Trisomy 8 is unusual, in that all cases were due to a meiotic error, which stands in contrast to somatic errors being almost entirely the basis of mosaic trisomy 8 that is diagnosed postnatally. Uniparental disomy appears not to be a causative factor in miscarriage (Shaffer et al., 1998; Smith et al., 1998b; Robinson et al., 1999).

Phenotypes of the Embryo/Fetus.

An embryo or fetus may or may not be identifiable in the products of conception collected at the time of spontaneous abortion due to chromosomal abnormality. Severe growth disorganization can be graded according to whether there is complete absence of any detectable embryonic parts, a tiny nubbin of tissue without recognizable embryonic landmarks, or an embryo in which cephalic and caudal poles can be distinguished (Philipp et al., 2003). The triploid embryo in Figure **23–4** is very deformed but with recognizable face, trunk, and limbs (but not as severely distorted as the trisomy 8 embryo in Fig. **23–2**). Seller et al. (2004) describe the only known case of (presumed) nonmosaic trisomy 2, a very severely deformed fetus that survived to 12 weeks gestation. Warburton et al. (1991) provide a graphic catalog of embryonic/fetal phenotypes from their material of about 1300 karyotypically abnormal spontaneous abortuses collected over a 12-year period in New York state, and we have mentioned earlier the illustrations of aneuploid embryos in Philipp et al. (2003). What actually leads to expulsion of the conceptus from the uterus may be the declining vascular and endocrine function of the placental tissue, with decidual necrosis (that is, death of tissue) finally causing uterine irritation and contraction (Rushton, 1981). The underlying process of decline, or at least a contributory factor, may be accelerated apoptosis: Qumsiyeh et al. (2000) observed a higher apoptotic index in villi of the abortus with an abnormal versus a normal karyotype.







Figure 23–4
A triploid (69,XXY) embryo. The face has no landmarks other than eyes and a single opening. The anterior trunk is open, with the heart and liver visible. Spontaneous abortion occurred at 18 weeks gestation, but the length is that of 6 to 7 weeks gestation. The disrupted tissue at the neck was the site of biopsy for the cytogenetic analysis.

Twinning.

If an abnormal twin dies, the normal twin may ensure continuation of the pregnancy, and only a parchment-like vestige (fetus papyraceus) remains, preserved in the uterus along with the normal twin. A “vanishing twin” has plausibly been proposed in the study of a pregnancy in which two cell lines were identified at CVS, 46,XX and 47,XY,+9. Amniocentesis gave a 46,XX result, and a normal girl was subsequently born. Analysis of a fibrotic area of the placenta gave the same two karyotypes, 46,XX and 47,XY,+9 (Falik-Borenstein et al., 1994). The likely explanation is that a 47,XY,+9 co-conceptus died, and the fibrotic placental tissue was the only remnant.

An extremely rare observation is the trisomy that would otherwise lead inevitably to early miscarriage, but in which a monozygous euploid co-twin allows some ongoing in utero survival. These cases may result from a very early postzygotic event that generates a trisomic cell line (or which generates a normal cell line from a trisomic conceptus), and the trisomic co-twin, among other devastating defects, fails to form a heart (“acardius”). The normal euploid co-twin provides the blood circulation to the abnormal fetus (“twin reverse arterial perfusion”). This scenario has been reported with trisomies 2 and 11 (Blaicher et al., 2000), and we have seen one case due to trisomy 3.

Fetal death in utero, perinatal death

Concerning mid-trimester loss, which, coming between miscarriage and stillbirth, may be referred to as fetal death in utero (FDIU) or in utero fetal demise (IUFD), chromosome abnormality may be present in about a half, although at this stage it is the “viable” rather than the invariably lethal aneuploidies that come to light (Howarth et al., 2002). Array methodology enables the detection of subtler imbalances, as Le Caignec et al. (2005) demonstrate in a series of polymalformed fetuses, the pregnancies terminating naturally or due to medical intervention. Similarly, a fraction of pregnancies going through to term, or at any rate to the third trimester, but with the baby stillborn or dying in the early neonatal period (perinatal death), are due to chromosomal abnormality, whether this be full or partial aneuploidy; a common representative of this group is trisomy 18. Again, array-CGH may increase the detection of chromosome abnormality or allow diagnosis when classic chromosomal analysis has been unsuccessful (Raca et al., 2009). Among liveborn babies, only 1 in 250 has an unbalanced chromosome abnormality on standard karyotyping (see Table 1–3 in Chapter 1). Thus, there has been a very effective selection against those conceptions that were abnormal (Fig. **23–5**).

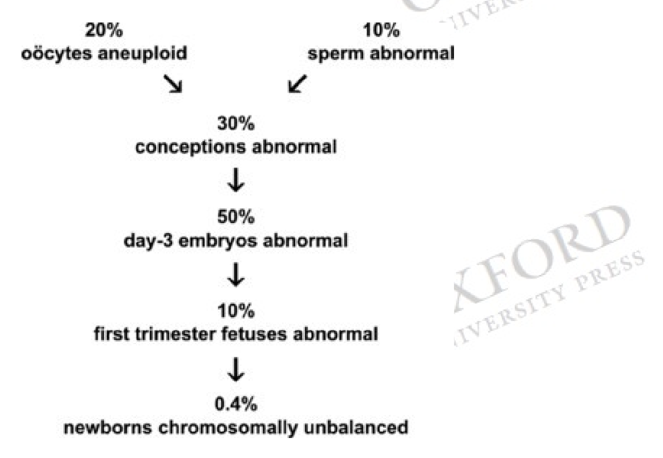




Figure 23–5
The frequency of chromosome abnormalities at gametogenesis and during pregnancy, demonstrating the effectiveness of selection against aneuploid states. The figures given for gametes through to embryos are very approximate, and considerable individual variation is probable. The oöcyte percentage varies very considerably according to maternal age. The day-3 embryo percentage, drawn from in vitro fertilization data, may exaggerate the true picture in vivo. The figures for fetal and newborn abnormality are quite accurate.

Recurrent pregnancy loss[5]

Do some couples, themselves karyotypically normal, miscarry on the basis of a predisposition to produce aneuploid conceptions? Ulm (1999) reviews theoretical possibilities through which such a risk might apply: recessive genes, parental chromosome abnormality, gonadal mosaicism, satellite association, and maternal age. A further suggestion, that of maternal skewing of X-inactivation, has since been excluded as a cause (Warburton et al., 2009). A case exists also for the basis of some recurrent aneuploid miscarriage being simply one of randomness, in the setting of a high background rate of aneuploidy in humans, with increasing maternal age the only clear predisposing factor. A common event is common, and not uncommonly it may happen more than once.

Addressing this question, Robinson et al. (2001) provided data from a study group of 54 couples having had 2–4 spontaneous known aneuploid/polyploid abortions, 122 abortions in total. The mean maternal age was 38 years. The distribution and frequency of karyotypes essentially did not differ from the data of a comparison group of 307 single miscarriage cases. Upon stratification for maternal age, a similarity of proportions of karyotypes in recurrent versus single abortions within each age group held up, and likewise upon comparison with an earlier analysis by Hassold et al. (1993). Trisomies in the Robinson study accounted for 80%, and the four most commonly represented abnormalities were trisomies 15, 16, and 21 (14%, 15%, and 8%, respectively) and triploidy (13%). A few couples had a repeat of the same trisomy, but these observations were not made more often than might have been expected by chance. In those cases in which the parental origin of the trisomy could be determined, the great majority, around 90%, were maternal. And yet, although the supposition of randomness and maternal age as the important contributors to recurrent miscarriage is given considerable weight by these data, to the contrary, a small effect indicating a predisposition to aneuploidy recurrence actually was discernible in Warburton's review of a very large body of prenatal diagnostic data (see p. [link]).

A somewhat different picture emerged from studies from Pellicer's group, in which the end-point of observation was FISH analysis of the embryo at PGD, from a patient population having previously experienced multiple miscarriage. In an initial report, nine couples were studied, the women of ages 30–35 years, having had on average four previous miscarriages, and who had presented for IVF with ICSI and PGD as a means to choose chromosomally normal embryos (Pellicer et al., 1999). Of 72 embryos biopsied at day 3 for karyotyping, 66 were analyzable, and in 35 (53%) an abnormality was found using FISH for chromosomes 13, 16, 18, 21, 22, X, and Y. A control group of similar age (10 women, ages 30–36) had an abnormality rate of only 19%, but a group of six older women (ages 37–41) had a comparable degree of abnormality (46%) to the miscarrying patients. (The controls were drawn from women of presumed normal fertility presenting for PGD for mendelian indications.) The chromosomal abnormalities included nullisomies, monosomies, trisomies, and tetrasomies for various of the five autosomes, and 45,X, 47,XXX and 47,XYY. As with the observations of Robinson et al. earlier, there was no apparent tendency for the same aneuploidy to recur.

A tentative conclusion is that a fraction of recurrent abortion may be explained by a predisposition to recurrent aneuploidy, and that this effect is more apparent in younger women. Further work by Pellicer's group (Rubio et al., 2003) has continued to support these findings, and the figures set out in Table 23–1 show notably higher frequencies of aneuploidies for the tested chromosomes in embryos of those women suffering recurrent miscarriage. The synaptonemal complex gene, *SYCP3*, appeared a good candidate as a basis for recurrent miscarriage, and Bolor et al., (2009) studied two women, heterozygous for a mutation in *SYCP3*, each having had three miscarriages, and no normal pregnancies. The case for *SYCP3* was, however, not supported in subsequent work from Mizutani et al. (2011).

Table 23–1. Frequencies of Embryo Aneuploidies for Certain Chromosomes in a Cohort of Women Having Had Recurrent Miscarriage (467–559 Embryos Analyzed), Compared with a Presumed Normally Fertile Cohort (104–202 Embryos)

	RECURRENT MISCARRIAGE		COMPARISON GROUP	
ANEUPLOID CHROMOSOME OF EMBRYO	⟨37 YEARS	≥37 YEARS	⟨37 YEARS	≥37 YEARS
13	20	19	7	21
16	24	29	7	16
18	10	12	5	15
21	23	27	9	30
22	18	25	5	14
X, Y	11	12	7	15

Notes: The two major classes of aneuploidy were autosomal monosomy and autosomal trisomy. Figures are percentages. Within each clinical category, distinction is made between women below age 37 years and those 37 and over. The rates of aneuploidy for chromosomes 16 and 22 show the most notable differences between the two cohorts, approximately two- to three-fold, across both age groups. For the other autosomes (13, 18, 21), the marked differences are confined to the women ⟨37 years. The comparison cohort comprised women having preimplantation genetic diagnosis for X-linked mendelian conditions.

Source: From Rubio et al. (2003).

It may seem counterintuitive, but for some couples who have suffered multiple miscarriage, it may be that an aneuploid abortion indicates a better chance for a normal live birth in a subsequent pregnancy than when a miscarriage is euploid (Ogasawara et al., 2000; Carp et al., 2001). Aneuploidy of the abortus may be *less* often observed in couples who have had a large number of miscarriages, sometimes into double figures, than among those who have had fewer. The probable reason, in such cases, is that a chromosomally normal miscarriage reflected an underlying maternal factor that would apply to all pregnancies, whereas aneuploidy at least offers the hope that better fortune might attend the next ovulation. But this basis is not universally applicable. Bianco et al. (2006), in a preliminary study, showed an increase in the likelihood for an aneuploidy to be found at prenatal diagnosis, in women who had had previous miscarriage, and this risk increased with the number of miscarriages.

Couples with rearrangements.

For the small group of people who are heterozygous for a chromosomal rearrangement, pregnancy loss may of course occur with a much higher frequency, and this briefly stated fact is the basis of much of what is written in this book. In about ½% of couples who have had more than two spontaneous abortions, and in a purely obstetric-

gynecologic referral base in which a karyotype is done as a first-up investigation, one or other of the couple is a translocation or inversion carrier (Simpson et al., 1989). Somewhat higher fractions (2%–5%) reported from other studies may reflect a degree of patient selection. These chromosomal rearrangements are typically of sufficient size to be readily detectable at standard karyotyping; and typically of sufficient size, that imbalanced combinations will lead, very frequently, to inevitable miscarriage. An actual example of a chromosomally unbalanced pregnancy leading to spontaneous abortion in the first trimester is shown in Figure 5–10 (in Chapter 5); this was the third miscarriage out of three pregnancies for the couple, the wife being a t(13;16) carrier. Cryptic translocations can also be associated with a risk for miscarriage (Diego-Alvarez et al., 2007). The risk for miscarriage will depend upon the particular characteristics of the rearrangement. As a global figure, for couples, one of whom carries a reciprocal translocation, there is an increased odds of three to fourfold, compared to chromosomally normal couples who have had repeated pregnancy loss, to have a subsequent further miscarriage (Ozawa et al., 2008). In contrast, the risk for a viable unbalanced form is very low in this group (Barber et al., 2010b).

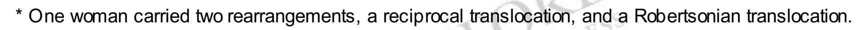


As well as translocations and other autosomal rearrangements, sex chromosome abnormalities may be identified in couples presenting with recurring pregnancy loss. Table 23–2 sets out the karyotypes seen in a Portuguese clinic population (Kiss et al., 2009). While this was a small series, the spread and prevalence of chromosome abnormalities is quite similar to the findings at infertility investigation (cf. Table 23–3).

Table 23–2. Chromosome Abnormalities in a Series of 108 Couples Having Had Recurrent Pregnancy Loss



KARYOTYPE	NO.
Sex Chromosomal	
45,X/46,XX	2
47,XXX/46,XX	1
47,XXY/46,XY	1
47,XYY/46,XY	1
Autosomal	
Simple rcp	3*
rob	2*
inv (pericentric)	1



* One woman carried two rearrangements, a reciprocal translocation, and a Robertsonian translocation.

Source: From a Portuguese clinic study 1975–2008; Kiss et al., 2009.





Cytogenetics of infertility

Infertility is defined as the inability to achieve conception, or the inability to sustain a pregnancy through to live birth (the latter known also as "infecundity").[6] Certainly, it is common, affecting about 15% of couples. It is worth emphasizing that infertility is to be seen in the context of the couple, not necessarily of the individuals separately. An oligospermic man may be fertile with a "superfertile" female partner, but not with a woman of average fertility, for example. Many causes of infertility exist, involving the male (Skakkebæk et al., 1994) and the female (Healy et al., 1994) partner, and a fraction of these are presumed to be genetically determined (Layman, 2002), and with demonstrable chromosomal causes seen in a minority. Sex chromosomal defects include XXY and XXY/XY in the male, typically presenting with azoöspermia and occasionally severe oligospermia in the nonmosaic state; and Turner syndrome and its variants in the female. The common Yq microdeletion is dealt with later. The XX male and XY female are rare (Chapter 20). Autosomal abnormalities are infrequently seen as a cause of infertility. The reciprocal translocation (especially when an acrocentric is involved) and the inversion may be associated, though infrequently, with severe hypospermatogenesis and moderate to severe oligospermia (Chapters 5 and 9). Robertsonian translocations are occasionally associated with infertility in the male or, less often, the female (Chapter 7). Translocation between a sex chromosome and an autosome is a rarely identified cause of infertility (Chapter 6). Complex rearrangements (Chapter 12) and rings (Chapter 11) typically present an insurmountable obstacle to cell division in the spermatocyte, resulting in azoöspermia; oögenesis is more robust.

The frequency of karyotypic abnormality in couples with infertility depends considerably upon the criteria of ascertainment, and quite wide ranges of figures have been produced. Couples presenting to ICSI programs might be supposed typically to manifest a male factor infertility; but van der Ven et al. (1998) were surprised to discover that female partners had about as many chromosomal abnormalities (X aneuploidy, reciprocal and Robertsonian translocations, inversions) as did the males, in a series of 305 couples presenting for ICSI; the experience of Meschede et al. (1998a) was not dissimilar. In an Italian series of 2710 infertile couples, more men were identified with a chromosome abnormality in those who had had ICSI, compared with those having standard IVF, and the least seen in those men in whom simple intrauterine insemination (IUI) was considered appropriate; no such differential applied to the women (Riccaboni et al., 2008).

A large French study (Gekas et al., 2001) brought together all the ICSI programs in France over a 3-year period and included some 3208 individuals, 2196 men and 1012 women, who had come forward as candidate couples for ICSI. Gynecologic causes of infertility had been excluded. Each individual had at least 20 metaphases examined. Sex chromosome mosaicism at a level of (10% was categorized as "minor." In the men, 6% showed a chromosomal abnormality, and in the women (excluding probably insignificant minor sex chromosome mosaicism), 2%. This latter figure may again seem surprising, given that the need for ICSI had been based upon the male partners' infertility. Certainly, in the fraction of their patients with simple "fertilization failure," cause unexplained, the proportion of women with a chromosome abnormality was rather greater, at 6.4%; but it remains that 2.6% of the women had an abnormal karyotype even when a basis of the infertility had been determined in the male partner. Support comes from a stringently conducted study in which an age-matched control population had less low-level 46,XX/45,X mosaicism (45,X in at least two cells) than women presenting for an ICSI procedure (Morel et al., 2002b). We return to the comment earlier about infertility being a couple condition.

The abnormalities included numerical and structural sex chromosome abnormalities, reciprocal and Robertsonian translocations, inversions, and other structural abnormality (Table 23–3). The French group compared their own data with ten other similar series, and their figures of about 6% and 5% for male and female karyotypic abnormality are close to the averages of about 5% and 4%, respectively. The figure is rather higher (16%) in men presenting with azoöspermia. Considering just autosomal translocations, and in relation to the nature of the infertility, Stern et al. (1999) noted the rate of balanced rcp and rob carriers to be 3% in 219 couples (both partners tested) who had failed more than 10 embryo transfers, and 9% in 130 couples who had 3 or more consecutive first-trimester abortions. (In one couple from the latter group, both were translocation carriers; see Fig. 12–6 in Chapter 12).

A rare complexity.

A most remarkable coincidence leading to infertility in a young woman is described in Kuechler et al. (2010). Her father was heterozygous for a mutation in the *FSHR* (follicle stimulating hormone receptor) gene, which is located at 2p16.3; and her mother carried an apparently balanced translocation, t(2;8)(p16.3;p23.1), but which in fact had a microdeletion at the 2p16.3 breakpoint, demonstrable on microarray. This microdeletion removed two exons of the *FSHR* gene. The daughter inherited this translocation, plus the paternal mutation, and in consequence, the former "unmasked" the latter, no normal FSHR was produced, and folliculogenesis was arrested.

Factors in the female

Fertility in the 46,XX female begins to fall in the mid-thirties, as the ovarian reserve dwindles. The average maximum number of 300,000 ovarian follicles is reached in midfetal life, at 18–22 weeks gestation, falling to 180,000 by age 13 years, 65,000 by 25, 16,000 by 35, and with less than 1000 remaining at the age of menopause (Wallace and Kelsey, 2010). This fall parallels both the increase in risk for trisomy, and an increasing failure in IVF implantation from the mid-thirties (Spandorfer et al., 2000). An important age-related factor may be a decline in the functional competence of the meiotic spindle, compromising chromosomal distribution and leading to the generation of aneuploid gametes (p. 40; Fig. 3–7).

Various sex chromosomal abnormal states, mostly mosaic and containing a 45,X cell line, account for—or are at least associated with—a number of cases of female *infertility*; autosomal abnormalities are less frequent. Some illustrative karyotypes are listed in Table 23–3. In some, the infertility is primary (there has never been a period of fertility), and in others, secondary (following a previous fertile period). In a Malaysian study, Ten et al. (1990) karyotyped 117 women with primary amenorrhea, who had previously been investigated for other causes, and one third had a sex chromosome abnormality. They were classified as follows: X aneuploidies (8%), X structural abnormalities (7%), presence of a Y (14%), and presence of a gonosomal marker chromosome (2%). Six women were mosaic, all having a 45,X cell line. Secondary infertility may be due to premature ovarian failure (POF), and Devi and Benn (1999) studied 30 women with unexplained secondary amenorrhea under the age of 40 years. Four (13%) had chromosomal abnormalities: an Xq isochromosome, Turner syndrome mosaicism (45,X/46,XX), an X-Y translocation, and an X-autosome translocation[7] (the fragile X premutation as a cause of POF is discussed on p. 248). Failure of the meiotic apparatus, with no formation of the first polar body, may be a rare cause of female infertility, and possibly due to an autosomal recessive gene (Neal et al., 2002; Schmiady and Neitzel, 2002).

A Robertsonian translocation may be an uncommon association with *recurrent pregnancy loss* (Kawano et al., 1998), as may the 47,XXX state (Itu et al., 1990). Kuo (2002) systematically studied 1010 couples having had recurrent loss and found two women with low-grade trisomy 21 mosaicism, each of whom had had at least one trisomy 21 abortus. Older (≥35 years) women who have had three or more miscarriages have an increased risk for a further loss, and trisomy is by far the most probable cause of this, being seen in about 80% of cases (Marquard et al., 2010).

In women who have suffered *recurrent implantation failure* at IVF treatment, in spite of having had a considerable number of embryo transfers, the rate of nonmosaic autosomal translocation carriers in one series of 65 women who had ≥15 failed transfers was 8%, two being sisters with the same translocation (Raziel et al., 2002). (Compare with the 3% translocation figure based upon rather larger numbers, and testing both of the couple, in Stern et al., 1999, noted earlier.)

Factors in the male

Fertility is not necessarily synonymous with normospermia and, as mentioned earlier, a man with oligospermia[8] may be fertile with a woman of "superfertility" (Krausz and McElreavey, 2001). Nevertheless, much couple infertility is associated with diminished sperm production in the male, and a fraction of this is associated with an abnormal karyotype (Table 23–3) (Gekas et al., 2001). In men presenting with azoöspermia or oligospermia, numerical and structural gonosomal abnormalities (mostly XXY and Y rearrangements) and structural autosomal abnormalities (mostly reciprocal and Robertsonian translocations) are identified in 3%–13% (De Braekeleer and Dao, 1991; Meschede et al., 1998a; Stuppia et al., 1998; van der Ven et al., 1998; Causio et al., 1999; Dohle et al., 2002; Elghezal et al., 2006). Rare observations include X;autosome translocations, and the small isodicentric 15 and other small marker chromosomes (Eggermann et al., 2002; Ma et al., 2003; Wang et al., 2009d).

X chromosome abnormalities.

The XXY state is the most frequently observed classical karyotype; some of these may be, and more especially in men with extreme oligospermia rather than azoöspermia, very low-level XY/XXY mosaic (Elghezal et al., 2006). Otherwise, mosaicism with a 45,X cell line, 45,X/46,XY, is often associated with infertility (Newberg et al., 1998), as also may be 45,X/47,XYY (Dale et al., 2002), and true XX/XY mosaicism, or chimerism (Sugawara et al., 2005). In some instances, if there is a residual spermatogenesis, artificial reproductive technology might enable fatherhood. The "XX male" is discussed on p. 335.

Y isochromosomes.

The typical Y isochromosome, 46,X,i(Y)(p10) or 46,X,i(Y)(q11), removes Yq-located spermatogenesis loci; the condition is seen in nonmosaic and 45,X/46,X,i(Y)(p10) mosaic forms. While abnormal genital and possibly neurodevelopmental phenotypes may be associated with this karyotype (see p. [link]), here we are discussing the otherwise normal male presenting with infertility. The typical basis of this may be "Sertoli-only syndrome," in which the testis lacks germ cells (Lin et al., 2005). While testicular extraction may sometimes enable sperm retrieval in this syndrome, we know of no cases of success in men with a Y isochromosome. We have seen a man, tertiary-educated, with nonmosaic 46,X,i(Y)(p10), in whom attempted testicular aspiration of sperm was unsuccessful (had IVF with ICSI been possible, PGD to select for an XX embryo would have been considered). The isodicentric Y isochromosome with a distal Yp breakpoint (and thus two copies of Yq) is a very rare form, and similarly related to infertility (Codina-Pascual et al., 2004).

Yq microdeletions.

The most frequent chromosomal cause of male infertility resides in a Y chromosome microdeletion, with particular reference to the AZF (azoöspermia factor) regions in Yq11, wherein certain spermatogenesis factors have their loci (see Fig. 6–1 in Chapter 6). The fraction varies according to patient selection, and when other causes of oligospermia/azoöspermia have been excluded, the proportion due to AZF deletion reaches 10%–20%. There is a large literature on this subject, reviewed in Foresta et al. (2001). While the initial discovery had been made by cytogeneticists (Tiepolo and Zuffardi, 1976), these Y-deletions are mostly not detectable cytogenetically, and are routinely analyzed using molecular methodology. There are three main AZF regions, a, b and c, and deletions in one or more region can impair spermatogenesis or lead to its complete failure. The most commonly seen deletion involves the AZFc region, in Yq11.23, the causative mechanism being similar to that described in Chapter 19, with inappropriate apposition of duplicons (Kuroda-Kawaguchi et al., 2001). AZFc contains the *DAZ*—deleted in azoöspermia—multigene family, the products of these duplicated loci being important (but not necessarily crucial) spermatogenesis factors. As a rule, AZFa or AZFb deletions are more severe in their effects than AZFc. Different causes for disordered spermatogenesis may coexist in an individual, and Jaruzelska et al. (2001) point to the need for cytogenetic studies, bringing to attention cases in which 45,X/46,XY mosaicism may have had an additive effect along with an AZFc deletion.

A male child conceived from a father with a constitutional Yq microdeletion would very likely have similar infertility (although, as noted later, some men with a Yq deletion may retain fertility). Komori et al. (2001) formally demonstrated that a man with a del(Yq) on blood karyotyping could transmit the deletion, in showing the deletion actually to be present in sperm, as did de Vries et al. (2001) in all of seven infertile men with deletion of the *DAZ* gene cluster. The observation of the same deletion in the sons of men who had conceived via ICSI confirms the reality of vertical transmission (Cram et al., 2000: Mau Kai et al., 2008). The reduction in fertility may be relative, at least for AZFc deletions, and at a younger age, and perhaps with a partner of "excellent" fertility, a man with a deletion may father children with no obvious difficulty (Krausz and McElreavy, 2001). Chang et al. (1999) report the example of an azoöspermic 63-year-old man with a *DAZ* deletion, but who had been fertile in his younger days, having had five children from when he was 25 to 38 years of age. His four sons all had the deletion, and the three of them tested (ages 24–37) were oligospermic or azoöspermic.

Translocation carriers.

In the setting of a balanced rearrangement, gametogenesis in the male heterozygote appears more vulnerable than in the female to the complexities imposed by a chromosomal abnormality, and infertility occasionally results. An important element in this male vulnerability may be the integrity at meiosis of the X-Y bivalent, synapsing and recombining at the pseudoautosomal regions at the tips of Xp and Yp—the “sex vesicle” (p. 129). Unpaired or aberrantly associating autosomal segments, particularly of the acrocentric chromosomes, might disturb this integrity, leading to disruption of spermatogenesis (Guichaoua et al., 1990; Oliver-Bonet et al., 2005; Vialard et al., 2006). Another element may be impaired synapsis of homologous segments in the normal and the rearranged chromosomes, which of itself prevents further progress in gametogenesis, and spermatogenesis may be more sensitive to this obstacle than is oögenesis (Hale, 1994; Oliver-Bonet et al., 2005). Pinho et al. (2005) undertook testicular studies on a man with a de novo 46,X,t(Y;1)(q12;q12), demonstrating reduced Xp/Yp pairing, and showing that spermatogenesis had arrested at meiosis I, and that the germ cells had undergone apoptosis. If spermatogenesis is retained, a compromised testicular environment due to the presence of a translocation may nevertheless, of itself, predispose to the production of diploid sperm (Egozcue et al., 2000).

Not only may the contained genetic material of the spermatozoön be faulty from the translocation heterozygote but also the “container.” Baccetti et al. (2003) studied by electron microscopy the sperm of an infertile man with a de novo t(10;15)(q26;q12) and showed essentially all sperm to be structurally abnormal. The acrosome was absent or abnormal, cytoplasmic residues were frequent, the mitochondria were often swollen, and axonemal structures were poorly formed. One might have considered that “spermatogenesis genes” on chromosomes 10 and 15 could have been compromised due to being in the monosomic or trisomic state, which then led to these structural defects in the unbalanced sperm. Yet about a third of sperm had a balanced chromosomal complement. Why were not a third of sperm ultrastructurally correspondingly normal? There is more to be learned about the genetics of spermatogenesis.

Notwithstanding the above, it remains true that fertility is usually unimpaired, or scarcely impaired, in male translocation heterozygotes. In their study of just over 10,000 sperm donors, all of proven fertility, the frequency of men carrying reciprocal and Robertsonian translocations, and also pericentric inversions, did not differ with statistical significance from the general population (Ravel et al., 2006a). The semen indices of these men were within normal ranges. This epidemiology indicates that while it is true that a few heterozygotes may have impaired fertility, their numbers are too small to sway the figures of a large carrier population into statistical significance.

Sperm phenotypic defects associated with chromosome abnormality.

A syndrome of infertility associated with “large-headed sperm” is described in Benzacken et al. (2001a). Polyploidy may be the explanation here. Benzacken et al. studied infertile brothers with oligoasthenoteratozoöspermia, half of all sperm having the large-head phenotype. FISH analysis (X, Y, 18) in one brother showed all sperm cells to be diploid or polyploid (3n, 4n, and)4n). The basic fault may lie in a failure of the cell to cleave at the two meiotic stages and, with brothers affected, a genetic cause may reasonably be presumed. Similar cases were reported by Devillard et al. (2002), Lewis-Jones et al. (2003), and Perrin et al. (2008). In the Lewis-Jones study, three men had complete teratozoöspermia (all sperm with abnormal forms, such as double heads, large heads, multiple tails), and the frequency of chromosomal abnormality was very high, up to 100%.

Another type of sperm defect is the “tail stump syndrome,” in which the flagellum, the motor apparatus, forms abnormally. Ravel et al. (2006b) report this defect in infertile brothers, both of whom carried a balanced t(5;12). It may be that a “flagellum gene” was disrupted at one of the breakpoints.

Normal karyotype.

Among infertile men whose karyotype is normal, and whose sperm count is abnormally low, there is an increase in the sperm aneuploidy/diploidy rate, with the sex chromosomes being the most prone to exhibit disomy (Shi and Martin, 2001). This effect is more apparent in those men with severe oligospermia, and in those aged 40 and over (Asada et al., 2000). Vegetti et al. (2000) assessed the influence of sperm count and motility, and showed that both these indices correlate with the frequency of sperm disomy, testing chromosomes 13, 18, 21, X and Y. The observations at testicular biopsy in men with severe oligoasthenozoöspermia support this interpretation, with univalents or oligochiasmatic and achiasmatic bivalents frequently being seen (Egozcue et al., 2000). Men with severe sperm indices may have a slight increase in sex chromosome abnormalities in peripheral blood (as a representative somatic tissue), when a very large number (1000) of cells are studied; and this may suggest a more generalized vulnerability of cell division, both meiotic and mitotic (De Palma et al., 2005). As for men with actual azoöspermia, in whom sperm can be obtained only by testicular or epididymal biopsy or aspiration, the early data on fairly small numbers also show elevated disomy rates for some autosomes and the X and Y chromosomes (Martin et al., 2000; Burrello et al., 2002; Mateizel et al., 2002; Palermo et al., 2002; Gianaroli et al., 2005). Mutation in the *SYCP3* gene (see later) has been implicated as a cause of spermatogenic arrest (Miyamoto et al., 2003), and other spermatogenesis loci are known (Avenarius et al., 2009).

Considerations relating to in vitro fertilization

It was reasonable to have imagined that IVF-conceived babies might be more likely to suffer a chromosomal abnormality, given the artificial circumstances of their conception, and in particular being aware of the increased rate of abnormality in the sperm of oligospermic men (Pang et al., 1999; Griffin et al., 2003; Silber et al., 2003). But the observation is of little, if any, such risk. One of the largest and most stringent studies addressing this question comes from Australia, in which 6946 IVF babies born in the period 1991–2004 were compared with 20,838 controls (Halliday et al., 2010). The rate of chromosome abnormality in the IVF babies was 0.99%, compared with 0.97% in the non-IVF babies. Among chromosome abnormalities evident at birth (thus excluding some mosaics and sex chromosome imbalances), the respective rates were 0.69% and 0.80%. (Interestingly, the only category of malformation for which a statistically significant increase was discerned for IVF was in respect of “defects of blastogenesis,” comprising a group of typically severe abnormalities which arise in the first 4 weeks post conception.)

Other studies have examined the issue of ICSI versus standard IVF, and there is a suggestion that a difference in chromosomal risk, albeit small, might exist. Possibly, the risks for *(a)* sex chromosomal aneuploidy and *(b)* de novo structural rearrangement may be increased in the children of severely oligospermic men (who will of course have needed ICSI to achieve conception). In a large French study (Bonduelle et al., 2002), documenting an 11-year experience comprising 1586 ICSI pregnancies in which prenatal diagnosis had been done, de novo structural aberrations and sex chromosome anomalies were seen in 1.6% (cf. 0.5% in the general population). These comprised 10 sex chromosome aneuploidies (XXY, XXX, XYY, and X mosaicisms) and seven structural rearrangements (mostly *apparently* balanced translocations), along with 8 cases of autosomal aneuploidy (mostly trisomies 18 and 21) All the gonosomal cases involved the father being severely oligospermic, and this male factor, rather than the ICSI procedure of itself, may have been the basis for the increase; the abnormality rate (gonosomal and autosomal) in children of men with sperm counts)20 million per milliliter was only 0.24%. We may conclude that an additional risk of chromosomal abnormality for children conceived from ICSI is marginal and may apply only in the case of men with very low sperm counts.

> Karyotyping of the oligospermic man is prudently to be done before proceeding to ICSI (Bonduelle et al., 2002; Griffin et al., 2003). Bofinger et al. (1999) provided ICSI to a couple, the husband having severe oligospermia, and the wife being of older childbearing age. At amniocentesis, on the grounds of the mother's age, a 45,X/46,X,r(Y) chromosome constitution was discovered, and belatedly, the same karyotype was found in the father. The experience of Veld et al. (1997) is equally telling, concerning two men who both, having suffered reproductive misfortune following ICSI, turned out to have a Robertsonian translocation.

Epigenetic effects.

Fertilization occurring in vitro is occurring in an artificial environment. It may be that the delicate interplay whereby the epigenetic reprogramming of chromosomes is applied, according to parent of origin, is vulnerable in this artificial setting (De Rycke et al., 2002); and the question arises that children born from IVF could be at increased risk for an imprinting disorder (Amor and Halliday, 2008). This does indeed appear to be the case with respect to Beckwith-Wiedemann syndrome due to epigenetic error, perhaps more so in the case of ICSI having been employed, and the risk is severalfold that of the general population (Halliday et al., 2004). The case for Angelman syndrome is rather more tenuous, with essentially anecdotal reports by 2008 of just five affected children born, four from ICSI conceptions. Nevertheless, the fact of these three cases all being the category of epigenetic error, and given the rarity of this type, must raise a valid concern. The third imprinting disorder associated with IVF is the “maternal hypomethylation syndrome” (Mackay et al., 2006). Equally, the statistical weight of the thousands of unaffected IVF children is not to be discounted, and this points to a low statistical level of

risk.

Genetics and pathogenesis of hydatidiform mole

Hydatidiform mole is an abnormal pregnancy that can be considered, in a sense, as a male chromosomal disorder. The pathology and genetics are reviewed in Petignat et al. (2003) and Slim and Mehio (2007). Typically, there is either a completely paternal karyotypic origin (two haploid paternal sets, 2n = 46) or an additional male haploid set (two paternal and one maternal haploid sets, 3n = 69). The presence of two paternal chromosomal complements is referred to as "diandry." The chorionic villi undergo a degenerative change, forming fluid-filled sacs (hence *hydatidiform*; *mole* means mass). The characteristic appearance has long been recognized (Fig. **23–6**). The phenotype is marked ("complete mole") when the genetic origin is completely paternal, and attenuated ("partial mole") in the presence of a maternal haploid contribution. Most cases are sporadic, but a specific genetic cause in rare recurrent cases may be maternal homozygosity for a predisposing gene.

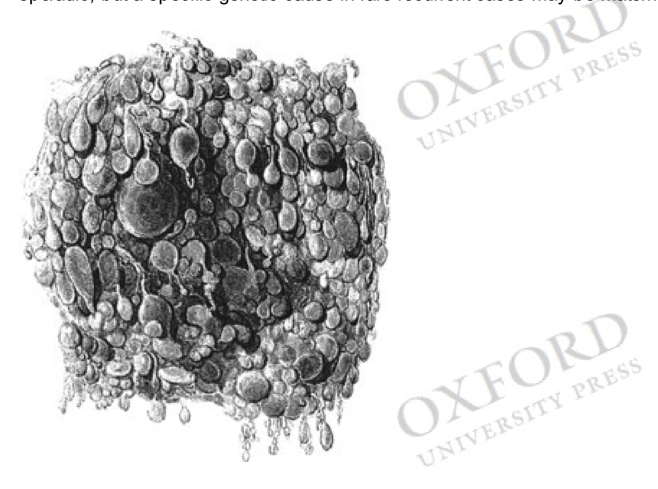



Figure 23–6
The appearance of hydatidiform mole, probably the first recorded depiction. (From Baillie, 1799.)

Complete mole

In the complete mole, there is placental tissue—swollen chorionic villi—but typically no embryo identified. The usual karyotype is 46,XX, looking, at first sight, like a normal female karyotype. This is due, in most, to a doubling (endoreduplication) of the chromosomal complement of a single 23,X sperm, while a minority are dispermic. In either case, there is no maternal chromosomal contribution. With the mole's nuclear genome being of entirely paternal origin, there is a total uniparental paternal disomy ("uniparental diploidy"). Moles due to doubling of a sperm chromosomal complement are entirely homozygous; in other words, they have a complete uniparental isodisomy. Complete mole occurs more often at the beginning and end of reproductive life in the female: it is more common in the early teenager and in women in their forties (Bagshawe and Lawler, 1982). Some diandric triploid molar pregnancies, when presenting earlier in pregnancy (before 8 weeks), may present a complete molar phenotype, rather than the partial mole usually observed (see later) (Zaragoza et al., 2000).[9] A small minority of moles have a biparental diploid genotype, one causative factor of which is apparently maternal homozygosity or heterozygosity for mutation in the *NLRP7* gene. This gene has a role in the acquisition of genomic imprinting as the ovum is produced, and it may also contribute to the ovum's safeguarding against polyspermy (Deveault et al., 2009; Hayward et al., 2009; Wang et al., 2009a; Slim et al., 2011; Qian et al., 2011).

The original explanation was that this endoreduplication of the sperm's chromosomal complement represented an attempted correction following fertilization of an "effectively empty egg," that is to say, an egg lacking a viable maternal pronucleus. This construction has been challenged, and Golubovsky (2003) doubts the existence of the empty egg. He proposes instead a schema whereby diploidization (a sort of "triploid rescue") in very early mitoses follows a dispermic triploid conception and generates 2n cell lines. If it is the maternal complement which is discarded in each cell (perhaps just two cells, following the first mitosis), the genotype is androgenetic: a mole follows.

The complete mole typically presents either at early ultrasonography with a "snowstorm" pattern of the placenta on ultrasonography, reflecting the swollen villi, or at first or second trimester vaginal bleeding. There is a widespread and marked hyperplasia of the trophoblast. Where diagnosis is made early, and curettage performed, some nonchorionic elements (yolk sac, capillaries, amnion) may be identifiable, and very occasionally, embryonic parts (Zaragoza et al., 1997; Petignat et al., 2003). Immunostaining for the $p57^{KIP2}$ protein (the $p57^{KIP2}$ gene being paternally imprinted) is a useful marker to discriminate between complete (staining-absent) and partial (staining-present) mole (Sebire and Lindsay, 2006). The incidence of complete mole is about 1 in 1500 diagnosed pregnancies, although regional/ethnic variations exist (Slim and Mehio, 2007). In Japan, the incidence has been falling, from about 1 in 400 in the 1970s, to 1 in 650 by the late 1990s (Matsui et al., 2003).

There is a small but significant risk of recurrence following one mole, the risk increasing if a woman has had more than one. Recurrences can be of either kind of mole, complete or partial. In a subset of patients, recurrent complete mole is unusual in being diploid and biparental, and as noted earlier, the *NLRP7* gene has been implicated; we await a full understanding of the mechanism whereby this effect is mediated.

Partial mole

An additional paternal haploid chromosome set is the basis of most cases of partial mole. This is triploidy, 69,XXX or 69,XXY (rarely 69,XYY), which may typically be the result of a normal ovum fertilized either with two sperm (dispermy) or with a diploid sperm, although other more complex scenarios are proposed (Zaragoza et al., 2000; Rosenbusch, 2008).[10]

Partial moles typically present as threatened, incomplete, or missed abortion, during the late first or early second trimester, the mean at 12 weeks. There is hydatidiform change of some villi, and the placenta is abnormally large. It is underdiagnosed and may occur in as many as 1 in 700 pregnancies (a figure Jeffers et al., 1993, derive from a review of all 2251 spontaneous abortions occurring in the catchment population of a Dublin hospital over a 3-year period during which there were 19,457 recorded pregnancies). Fetal development, in the very few cases proceeding far enough for this to be assessed, is characterized by a relatively normal growth pattern (McFadden et al., 1993). There appears to be little difference clinically between fetal development in 69,XXX and 69,XXY; the rare 69,XYY form has an earlier lethality (McWeeney et al., 2009). If the triploidy is confined to the placenta, it is possible for the pregnancy to proceed successfully to the birth of a 46,N child (Sarno et al., 1993).

Recurrences are on record, and a possible explanation is a genetically determined weakness in the zona pellucida of the ovum, which should act (the "zona reaction") to prevent more than one sperm penetrating. The double paternal contribution (diandry) is referred to as type I triploidy. Some cases of recurrence might reflect the effect of maternal homozygosity or heterozygosity for the *NLRP7* gene, as discussed earlier; this would be consistent with the observation that repeating moles can, in some cases, be of either type, partial or complete.

Placental mesenchymal dysplasia.

A possible clinical confounder, in that it rather resembles partial mole, but with apparently normal (or nearly so) fetal morphogenesis, is placental mesenchymal dysplasia (Ang et al., 2009). This is a form of mosaicism. The placenta is, in part, normal, and this part has a biparental (and diploid) genotype. The dysplastic part is also diploid, but of paternal uniparental origin. Fetal growth may be affected; a number have been associated with Beckwith-Wiedemann syndrome (Wilson et al., 2008; Jalil et al., 2009). The pathogenetic process, at least in the majority, occurs in the zygote, as the first mitosis gets underway, and lies in a failure of replication of the maternal chromosomal complement, while the paternal complement replicates normally. One paternal complement then joins with the maternal complement to form a biparental cell; the other paternal complement undergoes endoreduplication and produces a uniparental (androgenetic) cell. These two cells then give rise to mosaicism with two lineages, the biparental lineage substantially going on to form the fetus, and the androgenetic lineage responsible for the dysplastic component of the placenta. Intrauterine or neonatal death is a frequent outcome.

Genetic counseling

Recurrent miscarriage

People who have had one or perhaps two miscarriages generally do not come to a genetic clinic, and neither, as a rule, do they have cytogenetic analysis of the products of conception or an analysis of their own karyotypes. Their physician or obstetrician may have advised them that this loss will very likely be part of the 15% or so of all pregnancies that miscarry, and the chance of a successful pregnancy in the future would be good. But having had three miscarriages requires investigation (although others propose testing at an earlier stage; see next paragraph). To use the jargon, such couples have had multiple abortions, recurrent miscarriage, or recurrent pregnancy loss (or to put it in Latin, *abortus habitualis*). The usual gynecological investigations, and a chromosome analysis of the couple, should be done at this point. If a chromosomal rearrangement is identified, this will probably be the underlying cause (Gadow et al., 1991); but the possibility of a fortuitous discovery is not to be discounted. The precise nature of the rearrangement (consult the appropriate chapter), the reproductive history of any others in the family who have it, and the presence or absence of gynecological pathology allow one to make of judgment of its role in the etiology of the abortions. In the case of recurrent abortion due to a parent being a translocation carrier, Munné et al. (2000b) report that PGD can very substantially reduce the incidence of abortion, and if access to this technology is available, “translocation couples” may wish to consider this option.

The majority of couples will have a normal karyotype, 46,XX and 46,XY. In most centers, cytogenetic analysis of abortus material (an expensive and time-consuming procedure) is not routinely done, and so chromosomal normality or abnormality cannot usually be demonstrated. Some have argued that this policy should shift, and Stephenson et al. (2002) speak of “this unfortunate omission” compromising the management of couples presenting with recurrent miscarriage. For women in their late thirties, who have already had miscarriages, trisomy is the major underlying cause; analysis of products of conception can be useful in offering advice to these women, in that the actual cause can now be known, when trisomy is discovered (Marquard et al., 2010). Since the discovery of an aneuploidy can avoid the necessity for further investigation, Stephenson et al. argue that routine karyotyping would actually be cost-effective and have the further benefit of helping couples understand, and thus come to terms with, their reproductive failure, as Sánchez et al. (1999) have also suggested. Jobanputra et al. (2002) propose that a FISH panel for chromosomes 13, 15, 16, 18, 21, 22, X, and Y applied to uncultured abortus tissue would enable a relatively inexpensive screen, with culture proceeding only on those with an apparently normal result; more recently, molecular methodologies have been suggested in this setting (see earlier discussion).

A miscarriage due to aneuploidy actually implies a lower risk for miscarriage of a subsequent pregnancy than if the abortus is euploid. However, a previous aneuploid miscarriage may elevate somewhat the risk for subsequent aneuploidy at prenatal diagnosis. IVF with PGD might benefit some women who have had several miscarriages, although this is controversial (p. 429). Lathi et al. (2007) studied the karyotypes from products of conception in women who had miscarried, and they observed that a third of these would have been, in principle, detectable at PGD using 5-probe FISH, and up to three-quarters in 9, 10, or 12-probe FISH. Recurrent implantation failure (which could be seen, in a sense, as a very early form of miscarriage) is often due to chromosomal aneuploidy; in that case, PGD (or polar body analysis in those countries in which PGD-aneuploidy screening is prohibited) may be useful (Vialard et al., 2008) (and see Chapter 26).

We have yet to learn how frequently genes such as the *SYCP3* synaptonemal complex gene (see earlier) contribute to miscarriage. It may be that mutational analysis of this, and possibly other such genes, will become a part of the investigation of recurrent miscarriage. Women who carry a *SYCP3* mutation apparently (although proof is awaited) have a high risk to produce an aneuploid conceptus.

Fetal death in utero

Pregnancy loss in mid-trimester is less frequent than in the first trimester, and some may thus see a lower threshold for karyotyping the products of conception. In this case, Howarth et al. (2002) propose offering chorionic villus sampling or amniocentesis, rather than attempting culture of fetal tissue post delivery, in order to improve the chances of getting a definite chromosomal result.

Women of older childbearing age

Maternal age is an important factor in recurrent miscarriage. The meiotic apparatus of the oöcyte deteriorates with age; returning to Figure 3–7 (in Chapter 3), the reader can marvel at the disposition of the chromosomes in the eggs of the older women and appreciate how perfectly plausible it is that egg after egg could be aneuploid. The evidence from IVF points to a sharply increasing likelihood for aneuploid conception in women of older childbearing age (Verlinsky et al., 1999). Some instances of reproductive “bad luck” can seem like very bad luck, but, as mentioned earlier, this may still be the true explanation. Ulm (1999) describes her experience dealing with two couples having had several losses, expressing her and their frustration in not being able to provide a satisfactory explanation, nor to offer a precise recurrence risk. In the first case, following a normal child at the mother's age of 34, the next four pregnancies, in her late thirties, miscarried or were terminated. The first was not tested, and the next three were trisomic 22, 21, and 14, respectively. The sixth pregnancy, at age 40, tested 47,XX,+15 on direct CVS but normal on long-term CVS and at amniocentesis, with a biparental chromosome 15 pattern. The infant was developing normally as an 8-month-old. The second case concerned a woman having three miscarriages at age 36–37 years, which karyotyped as trisomy 22, 16, and 21 respectively.

We are not much able to ease Ulm's frustration, other than to point again to the high background rate of human aneuploidy, and to the major influence of maternal age. It is not easy to distinguish, just on the basis of history, which older couples are destined to experience reproductive misfortune. The counselor needs to recognize that many in this situation will go on to have successful pregnancies, but retaining quite some reservation that the risk may be “significant,” and perhaps “substantial,” for women who are getting into their late thirties or forties. For some, with concern that their reproductive years may be limited, IVF with PGD may seem an attractive option, but advice will need to be tempered by the evolving understanding of pregnancy outcomes from PGD for aneuploidy screening in this setting (p. 428).

Infertility

Infertility is common, and, in Western countries at least, about 15% of couples wishing to have a child are affected (Foresta et al., 2002). Intrinsic fertility cannot be restored in men with persistent azoöspermia associated with seminiferous tubule failure, and neither in women who have had ovarian failure. The counselor will need to understand how disappointing and indeed devastating this may be to some couples (sometimes one of them more than the other), and to be prepared to deal with this. Those for whom assisted reproductive technology may offer hope need to be well aware that this is not necessarily an easy path, and neither can success be guaranteed.

Among the catalog of investigative tests that are available, a karyotype is well up on the list. A grouping of experts from the Italian professional community has addressed the question of what tests should be done and when (Foresta et al., 2002). They propose that karyotyping should routinely be done in men with azoöspermia and oligospermia, and in the United Kingdom, karyotyping of men presenting for ICSI is “commonplace” (Griffin et al., 2003). Yq microdeletions should be checked for in men with nonobstructive

azoöspermia and severe oligospermia, but this is unlikely to be the cause in lesser degrees of oligospermia (⟩5–10 million/ml) (Foresta et al., 2002; Quilter et al., 2003). At present, sperm karyotyping is a discretionary investigation (if available).

Karyotyping should be routine in women presenting with primary ovarian dysfunction or recurrent miscarriage. Fragile X premutation analysis should be considered in women with premature ovarian failure (Streuli et al., 2009); a consideration here is the requirement for informed consent, given the other implications of making this diagnosis. Papanikolaou et al. (2005) pose the question, Is chromosome analysis mandatory in the initial investigation of normovulatory women seeking infertility treatment?—and answer in the negative. In other words, and having reviewed over a thousand infertile women with normal ovulatory cycles, the number and type of chromosome abnormalities detected differed scarcely from a normal neonatal population. A greater return came from karyotyping in women with secondary infertility.[11]

Infertility with parental chromosomes abnormal

If a chromosomal defect is discovered in one or other of the couple, this at least provides an explanation for the infertility and (according to the exact nature of the defect) may prevent the disappointment of undergoing pointless further investigation. In some, artificial reproductive technology may enable a normal/balanced gamete to be identified and retrieved, and used at IVF. Where this is impossible, artificial insemination or IVF using donor gametes offers an entrée to parenthood, and it may enable one of the couple to be a genetic parent.

Women.

In women with a sex chromosomal abnormality having oöcyte donation, endocrinological management may be necessary to "prime" the reproductive tract (Devroey et al., 1988). But if the internal apparatus is intact, success may well follow, as is rather notably illustrated by the patient reported in Chen et al. (2003b), who had a Turner syndrome variant due to an isodicentric X, and who produced triplets following donation. In some cases the woman's own mother, with whom of course she shares half her genes, has been the donor. Artificially stimulated ovulation has been attempted in one case of a chromosomal state associated with secondary amenorrhea. Causio et al. (2000) describe a 29-year-old woman with a 46,X,t(X;16) karyotype who had undergone premature ovarian failure, and in whom ovulation was then achieved by treatment with gonadotrophin releasing hormone and follicle stimulating hormone (GnRH and FSH). But no pregnancy resulted.

A small fraction of female partners of oligospermic men are found to have low-level sex chromosomal mosaicism. The reality is, according to the experience of Sonntag et al. (2001), that this does not compromise the course and outcome of ICSI.

Men.

In men with complete spermatogenic arrest, gamete donation may be considered. In those in whom the chromosome defect leads to oligospermia, rather than complete failure of spermatogenesis, IVF with ICSI is a possible means to achieve pregnancy, and PGD will often be appropriate. Otherwise, given the small increased risk for gonosomal aneuploidy following ICSI, a subsequent conventional prenatal diagnosis may appropriately be offered. *Translocations* and other rearrangements need to be assessed on their merits. A small (but growing) number of cases of fatherhood in men with *Klinefelter syndrome* have resulted from ICSI (p. 224). *Rare sex chromosome* abnormalities are judged individually. For example, a sperm study in the case of a man with sex chromosomal mosaicism (45,X/47,XYY), which gave normal findings, was instrumental in a decision not to have preimplantation diagnosis following an ICSI conception (Dale et al., 2002).

In the case of a *Yq microdeletion*, couples choosing the option of IVF with ICSI should know that a male child would be predicted to have, very probably, the same type of infertility (Foresta et al., 2001). Some might consider having PGD to ensure having a daughter; although Kim et al. (1998) comment that "interestingly, after genetic counseling, the decision to proceed with ICSI for the overwhelming majority of couples remains unchanged." Nap et al. (1999) assessed 28 such couples, and they interviewed the 10 counselors who had seen them, in six clinics in the Netherlands and in Belgium. A considerable majority of couples (79%) chose to continue with plans for ICSI, with only a few choosing donor insemination (7%), or opting out altogether (14%).

Infertility with parental chromosomes normal

Where the male has oligospermia, and if IVF with ICSI is to be attempted, there are the grounds (discussed earlier) for presuming a very slightly increased risk for de novo structural aberration or gonosomal aneuploidy. Sperm karyotyping is not routinely practiced as a basis for informing genetic counseling, although this is an area in which research is continuing and opinions are evolving (Griffin et al., 2003; Machev et al., 2005). It may be prudent to offer prenatal diagnosis for an ICSI-produced pregnancy. However, given the immense investment couples will have made to achieve the pregnancy, there may be reservation about proceeding to an invasive prenatal diagnostic procedure, even being aware of a possibly increased genetic risk. In this context, the data from Aytoz et al. (1998) offer reassurance. These Belgian workers compared outcomes in 576 ICSI pregnancies having amniocentesis (singletons) or chorionic villus sampling (twins), with 540 control ICSI singleton and twin pregnancies not having prenatal diagnosis. The fetal loss rates, and certain obstetric indices (preterm delivery, low birth weight), did not differ significantly between the two groups. Further, the data were not tending in the direction of a greater risk for miscarriage in the prenatal diagnosis patients: the odds ratios for fetal loss were 0.86 (amniocentesis) and 0.47 (CVS) compared to the controls. Nevertheless, in a German population, Meschede et al. (1998b) report that only 17% of a cohort of 107 women having undergone ICSI chose subsequent amniocentesis (or fetal blood sampling), the great majority preferring noninvasive ultrasonography or serum screening. This preference was more marked in those who had had genetic counseling prior to entering the ICSI program. In contrast, an Italian clinic recounted a very opposite figure, with 86% choosing invasive prenatal diagnosis (and 100% choosing ultrasound screening); these workers could see "no logical explanation for the great difference" (Monni et al., 1999).

The known or suspected risks for an imprinting disorder (Beckwith-Wiedemann syndrome, Angelman syndrome, maternal hypomethylation syndrome, as discussed earlier) in IVF-conceived children raise a question that it may be prudent to advise couples of these concerns, according to the accumulating understanding of these risks. Counselors working in the IVF clinic will want to maintain a watching brief.

Chromosomally abnormal children following pregnancy by donor insemination.

If a pregnancy achieved through gamete donation turns out to be chromosomally abnormal, should that donor continue to be used? Kuller et al. (2001) surveyed a number of reproductive endocrinologists and obstetrical geneticists to determine the current practice, with particular reference to trisomy 21 and monosomy X. It was clear that no consistent policy was followed. For chromosomal abnormalities generally regarded as being sporadic (or where any predisposition might reside in the recipient rather than the donor), it would seem unnecessary to remove that donor from the panel.

In vitro fertilization and multiple pregnancy

Twinning and higher multiple pregnancies are common in IVF, for the obvious reason that often more than one embryo is transferred following IVF, this being a standard policy to improve the chances for a successful implantation. The conservative number of transfers is two, so that if both embryos do implant, no more than twins will result (unless a single embryo might go on to produce monozygous twins). Some clinics transfer more, sometimes for the simple economic reason that if couples can only afford one IVF cycle and transfer, using three (or even more) embryos increases the chance of pregnancy. The disadvantage is, of course, that if most or all of the embryos implant, a high multiple pregnancy results. Whatever might be the risk for aneuploidy,[12] that risk will apply to each embryo individually, thus increasing the overall risk that one at least might be chromosomally abnormal. If both abnormal and normal fetuses are present, and diagnosed at subsequent amniocentesis, selective feticide of the chromosomally abnormal fetus may be chosen, or for a lethal aneuploidy (trisomy 13, trisomy 18) the parents may opt to continue the pregnancy in the expectation that the abnormal fetus will die (Sebire et al., 1997). One of the claims made for PGD is that single embryo transfers can be done with a better expectation of success, and a twin pregnancy avoided.

Hydatidiform mole

The risk of recurrence in a subsequent pregnancy is about 1% (Garrett et al., 2008), and recurrence can be either of the same (complete or partial) or of the other type. Ultrasonographic surveillance is advisable in a future pregnancy. Having had a second mole, the risk for a third is considerably higher, on the order of 20% (Berkowitz et al., 1994).

A few of the single cases, but possibly most of the multiple recurrences, may be due to maternal homozygosity or even heterozygosity for mutation in the *NLRP7* gene; the counselor will need to consult current literature as we learn more about this gene, and how it acts. These repeating cases typically show biparental inheritance, in contradistinction to the androgenetic basis of the majority of moles. Fisher et al. (2000) suggest that parental origin is worth establishing in those couples who might have been considering IVF with ICSI (to ensure entry of a single sperm), or PGD for ploidy diagnosis, as a means to diminish the risk in a subsequent pregnancy, since such approaches would be futile if the mole(s) had been biparental.

A major aspect of management is that the mole may undergo neoplastic transformation (gestational trophoblastic disease). With *complete mole*, the risk for the development of invasive mole is about 15%, and for the more dangerous choriocarcinoma, it is 3%. The risks are much less with *partial mole*, the respective figures being 0.5% and 0.1% (Seckl et al., 2000). These facts need to be borne in mind by those women choosing to attempt a further pregnancy.

We are unaware of any reports of recurrence of placental mesenchymal dysplasia.

Notes:

[1] Warburton suggests that this error rate conveyed an evolutionary advantage in former times: miscarriage due to aneuploidy led to a wider spacing of offspring, allowing a woman to devote scarce resources to a more manageable number of offspring, more of whom would survive to contribute their genes to the following generation. What was beneficial in a prehistoric society has been quite the opposite for many women, now hostage to their biology, in the present century (a concept not without several other examples).

[2] Strictly speaking, in utero life is divided into three periods: pre-embryonic (the first 2 weeks), up to formation of the primitive streak; embryonic (to the end of the 8th week) when the body forms and organs are constructed; and fetal (from 8 weeks to term), characterized by growth and changes in proportion rather than the appearance of new features. Often the word *fetal* is used loosely to refer to the entire period, and in IVF parlance (and in the present discussion) the word *embryo* is routinely applied in reference to the conceptus in the early cleavage stage during the first few days. “Conceptus,” in theory, applies to any stage, but it generally refers to early pregnancy. The conceptus at the one-cell stage—the fertilized egg—is the zygote.

[3] This expression refers to a conceptus in which a gestational sac and possibly a yolk sac exist, but no recognizable embryonic parts, or at most a “nubbin” of tissue. *Blighted ovum* and *anembryonic miscarriage* mean the same thing.

[4] The distinction between an embryo and a fetus (and see note 2) in this setting is not necessarily straightforward. Embryonic development may have arrested, and spontaneous abortion will be inevitable, but the pregnancy may continue for one or a few weeks (“missed abortion”), and using apparent gestational age would give a misleading impression. In this case, it is more useful to consider the developmental stage of the embryo in judging the effects of a particular aneuploidy (Philipp et al., 2003). For example, the triploid embryo (not fetus) shown in Figure **23–4** was retained in the uterus until 18 weeks, but development had arrested at the 7–8 week mark.

[5] Definition from the American Society for Reproductive Medicine (2008): Recurrent pregnancy loss is a disease distinct from infertility, defined by two or more failed pregnancies. When the cause is unknown, each pregnancy loss merits careful review to determine whether specific evaluation may be appropriate. After three or more losses, a thorough evaluation is warranted.

[6] Definition from the American Society for Reproductive Medicine (2008): Infertility is a disease, defined by the failure to achieve pregnancy after 12 months or more of regular unprotected intercourse. Earlier evaluation and treatment may be justified based on medical history and physical findings and is warranted after 6 months for women over age 35 years.

[7] X-autosome rearrangements associated with ovarian failure may reflect a consequence of breakpoints in one of the Xq21 critical regions (CR1), which bring autosomal “ovarian genes” under the influence of X-heterochromatin at Xq21. In other words, it may be a position effect, whereby autosomal genes for ovarian function are down-regulated due to epigenetic modification (Rizzolio et al., 2006).

[8] Oligospermia is defined as a sperm count of ⟨20 million per milliliter. Oligoasthenoteratozoöspermia includes the observations of poor motility (*astheno*) and an increased fraction of abnormal forms (*terato*). Severe oligospermia is a count of ⟨2 million per milliliter, moderate oligospermia is 2–5 million per milliliter, and mild is 5–20 million per milliliter. Azoöspermia is absence of sperm. A distinction is to be made between obstructive and nonobstructive azoöspermia; in the latter, the primary fault is a severe defect of spermatogenesis.

[9] A rare—or rarely recognized—scenario is that of confined placental mosaicism for molar and normal tissue, the infant being normal (Deveault et al., 2009). Photographs of the placenta in one such case give an obvious visual illustration of the mosaicism (Makrydimas et al., 2002). A possible mechanism is that the sperm underwent an inappropriate mitotic division to give two male pronuclei in the zygote, one of which fused with the female pronucleus (to give the cell leading to the normal child), and the other underwent endoreduplication (to produce a cell that gave the molar component). Or this may simply be the end result of chaotic mosaicism in the first few mitoses, with the two surviving cell lines happening one to be normal, and the other androgenetic.

[10] Very uncommonly, partial mole has a normal diploid karyotype, with biparental inheritance. One very rare association is with large autosomal trisomy, such as trisomy for almost all of chromosome 4 (Fritz et al., 2000).

[11] Secondary infertility refers to a couple having previously had a successful pregnancy, but who are currently unable to conceive.

[12] If donor eggs are used, it is the age of the donating woman that counts in determining the age-related aneuploidy risk.







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Chromosome Abnormalities and Genetic Counseling (4 ed.)

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Parental age counseling and screening for fetal trisomy

Chapter: Parental age counseling and screening for fetal trisomy
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THE MATERNAL AGE association in Down syndrome (DS) was known long before its chromosomal basis. In 1909, Shuttleworth wrote that "with regard to parentage … the outstanding point is the advanced age of the mother at the birth of the child … the next point that strikes one is the large proportion of Mongol children that are lastborn, often of a long family" (p. 662). He considered that either age or parity could be an etiologic factor. Subsequently, Penrose (1933, 1934) demonstrated that it was the mother's age that was the key factor. A powerful insight into the actual nature of the maternal age effect has been afforded by Battaglia et al.'s (1996) study in normal women, showing that the oöcyte's meiotic apparatus deteriorates with age.

Sherman et al. (1994) stated "… increasing maternal age is one of the most important factors in human reproductive failure, as well as being a leading contributor to mental retardation among live-borns." Hassold et al. (1993) commented that "The association between increasing maternal age and trisomy is arguably the most important etiologic factor in human genetic disease. Nevertheless, we know almost nothing about its basis"; and likewise Wolstenholme and Angell (2000) observed "… there is still no consensus of opinion as to how aneuploidy arises in man, and there is a surprising lack of understanding of the basic mechanism(s) of the well-established links to maternal age." Some suggested factors are outlined in Chapter 3 (p. 39).

The maternal age effect in DS—whatever it may be—has been considered to operate upon oögenesis, predisposing to nondisjunction of chromosome 21 predominantly at the first meiotic division.[1]

In more general terms, segregation of some other chromosomes is vulnerable to the maternal age effect; and, thus, "older women" who are pregnant run an increased risk for having a pregnancy with trisomies 13, 16, and 18, 47,XXX and 47,XXY, as well as trisomy 21. There is also a slight maternal age association with disorders due to uniparental disomy (Ginsburg et al., 2000), this point being discussed in more detail in Chapter 22. Advanced maternal age—or to use the preferable expression of Ginsburg et al. "mothers at advanced childbearing age"—is a common indication for prenatal diagnosis, although becoming less predominant due to the impact of maternal serum and ultrasound screening.

Paternal age generally does not usefully enter the equation, at least with respect to autosomal aneuploidies. Fathers of DS children are older than average, but simply because couples are usually of similar ages, a point determined some 75 years ago by Penrose (1934). In a study from the Atlanta and National Down Syndrome Projects, Allen et al. (2009) identified no association with paternal age per se. Concerning gametic studies in older men, numerous sperm analyses have been done, with somewhat conflicting findings (Robbins et al., 1997; Shi and Martin, 2000b; Eskenazi et al., 2002). Some have shown slight increases in some autosomal disomies, and some have shown increases in sex chromosome disomies, with XY disomy being more consistently noted. Other studies report no significant differences in at least autosomal abnormalities comparing older and younger men (one group even using testicular sperm from men in their eighties; Guttenbach et al., 2000). By no means is XY disomy always raised; for example, Luetjens et al. (2002) found, in their study of men under 30 and over 60 years of age drawn from general populations, no differences in XY disomy between the two groups. It is also to be noted that X-Y recombination (its absence being a risk factor for nondisjunction) occurs no less frequently in older than in younger men (Shi et al., 2002). In one study of men who had actually fathered a child with XXY Klinefelter syndrome, the frequencies of 24,XY sperm did increase with increasing age of the father, although it may be an uncommon individual predisposition, rather than an actual age-related factor, that was substantially responsible for this (Lowe et al., 2001; Eskenazi et al., 2002). As for structural rearrangements, a true paternal age effect is considered to exist, albeit at an order of magnitude less than the maternal/aneuploidy association (Sloter et al., 2004).

Risk Figures According to Maternal Age

How old is "older," and what is "advanced" maternal age at childbearing? Conventionally, the mid to late thirties is taken as the boundary. The risk curve for DS, the major condition of concern, begins to steepen at this period, although there is no sudden jump. Risk figures for individual ages with respect to this and other aneuploidies have been collected in various jurisdictions, and estimates refined according to certain statistical assumptions, and the information from these studies has long been used as the basis of preconceptional and prenatal genetic counseling. These data are also useful in screening programs for fetal trisomy (see later), the woman's age-related risk being an important datum to be included, along with the various laboratory test results, to derive her overall risk estimate.

For trisomies 13, 18, and 21, spontaneous abortion is more likely than for a normal conceptus. Thus, the prevalence of chromosome abnormality is greater at the time of prenatal diagnosis than at term, and we need access to stage-specific figures. Looking through these different windows of observation—at chorion villus sampling (10–11 weeks), at amniocentesis (about 15–17 weeks), at screen-triggered amniocentesis (may be closer to 20 weeks), and at term—the frequency of chromosomal abnormality, for a particular maternal age, progressively reduces. For trisomy 21, it is estimated that about 30% of all pregnancies existing at the time of CVS abort between then and term, and 24% abort during the period from amniocentesis to term (Table 24–1 and Fig. **24–1**). Trisomies 13 and 18 (and monosomy X) have high rates of fetal lethality, with the majority of pregnancies aborting. For XXX and XXY, in contrast, there appears to be very little selective loss in the latter part of pregnancy.

Table 24–1. Natural Fetal Loss Rates from Early Pregnancy through to Term, Estimated for the Three Major Autosomal Trisomies and X Monosomy			
ESTIMATED ABORTION RATE (%)			
CHROMOSOME ABNORMALITY	**FROM 10 WEEKS TO BIRTH**	**FROM 16 WEEKS TO BIRTH**	**FROM ~20 WEEKS TO BIRTH***
Trisomy 13	83	71	
Trisomy 18	86	74	32
Trisomy 21	31	25	10
Monosomy X	76	52	

Note: 10 and 16 weeks approximate to the stages at which CVS and amniocentesis are performed.

* Trisomy diagnosed at amniocentesis, following population-based maternal serum screening offered at 15–20 weeks.

Sources: From Spencer (2001), Snijders et al. (1995), Won et al. (2005), and Savva et al. (2006).

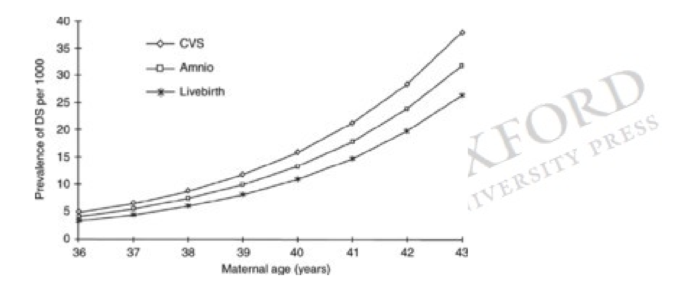




Figure 24–1
Prevalence of Down syndrome for maternal ages 36–43, at three “windows of observation”: the time at which CVS is done (about 10 weeks), amniocentesis (15–17 weeks), and at live birth. (From the study of Halliday et al., 1995, courtesy J. L. Halliday.)

These matters may be of particular importance to those women who, having had an abnormal result, nevertheless decide to continue a pregnancy. How likely is it that they will have a liveborn baby with the trisomy in question, or that fetal death in utero will supervene? Won et al. (2005) reviewed 392 women who had continued a trisomy 21 pregnancy, and 106 with trisomy 18; the diagnoses had been given somewhat later than might be usual, since these women had entered a public maternal serum screening program at gestations ranging from 15 to 20 weeks, with amniocentesis then offered to those who returned an increased risk result. For trisomy 21, fetal demise occurred in 10%, and for trisomy 18, 31% (Table 24–1). About a third of the trisomy 21 losses happened before the stage of viability (that is, 24 weeks), the comparable figure in trisomy 18 being 15%. In those pregnancies proceeding beyond 24 weeks, the losses were evenly spread according to duration.

Down Syndrome

The largest body of data to be collated for the age-related risk of trisomy 21 is that of Morris et al. (2002), who examined records from a 10-year period, 1989–1998, in England and Wales. We have used their material as the basis of the age-related live-birth figures to age 44 presented in Table 24–2, as probably the best available, although in fact the estimates for younger women (up to age 34 years) have been very similar in all studies, and quite similar in the 35–44 year age bracket (Morris et al., 2003). However, and contrary to earlier interpretations, the risk of having a baby with trisomy 21 does not increase from age 45 and older (Morris et al., 2005a). This might reflect a greater tendency to miscarry an abnormal fetus in women in their late forties and early fifties, or hypothetically a “meiotic robustness” in some women of this age who are yet able to achieve pregnancy. Estimates for the risks of detection of trisomy 21 at prenatal diagnosis, at the maternal ages at which the procedures would be done, are given in Table 24–3.

Table 24–2. Maternal-Age-Specific Risks for Trisomy 21 at Live Birth

MATERNAL AGE	PREVALENCE AT LIVE BIRTH		MATERNAL AGE	PREVALENCE AT LIVE BIRTH	
YEARS	‰	1 IN	YEARS	‰	1 IN
14	0.9	1108	34	2.3	430
15	0.4	2434	35	3.0	338
16	0.5	2013	36	3.9	259
17	0.6	1599	37	5.0	201
18	0.6	1789	38	6.2	162
19	0.7	1440	39	8.8	113
20	0.7	1441	40	12	84
21	0.7	1409	41	15	69
22	0.7	1465	42	19	52
23	0.7	1346	43	27	37
24	0.7	1396	44	26	38
25	0.7	1383	45 and older	34	30
26	0.8	1187			
27	0.8	1235			
28	0.9	1147			
29	1.0	1002			
30	1.0	959			
31	1.2	837			
32	1.4	695			
33	1.7	589			

Source: From Table 2 in Morris et al. (2002), the data up to age 44, and from Morris et al. (2005a), for age 45 and older. The figures to age 44 are based on data from just over 6 million births in England and Wales 1989–1998; the figure for 45 and older comes from a review of several sources internationally. Prenatal diagnostic data were included in this material, weighted according to the probability of survival to term. No trisomy 21 pregnancies were recorded at ages 11–13 (274 births) or at ages 53–55 (169 births). The per thousand figures (‰) are rounded.

Table 24–3. Maternal-Age-Specific Risks for Trisomy 21 Calculated at 10 Weeks Gestation (the Usual Time for CVS) and at 16 Weeks (Amniocentesis)

MATERNAL AGE*		GESTATION	
YEARS		10 WEEKS	16 WEEKS
20	1 in	800	1050
25		710	930
30		470	620
31		410	540
32		350	460
33		290	380
34		235	310
35		185	245
36		150	195
37		115	150
38		90	115
39		65	90
40		50	70
41		40	50
42		30	40
43		20	30
44		15	20

* Age at the indicated gestation.

Source: From Table 2 in Snijders et al. (1995). Figures are rounded.

Systematic calculations from non-Caucasian ethnic groups have come from Nigeria, China, Japan, South America, and Hawaii. Adeyokunnu (1982) showed, in Nigeria, no difference in incidence compared with Europeans; and in a study encompassing nine South American countries, Carothers et al. (2001) demonstrated incidence data and maternal age correlations very similar to those recorded from other jurisdictions. In Japan, Yaegashi et al. (1998) collected data from four clinics, comprising, in all, 5484 pregnancies of women 35 years and older. The risks for trisomy 21 (and for aneuploidies) overall were, on the face of it, somewhat less than in a European population. The raw figures did, however, fluctuate somewhat, with rather small numbers of affected fetuses at each age category. A question might be raised whether some cases could have escaped ascertainment by earlier screening and not otherwise recorded. It may be premature to suppose that aneuploidy rates could differ to any important degree between Japanese and other ethnic groups, a view that is supported by the observation otherwise of no significant differences in a Hong Kong population (Lau et al., 1998); but bearing in mind also data from Hawaii suggesting that, at older maternal ages, the DS rate *may* be somewhat less in a Pacific Island population (Forrester and Merz, 2003b). Taking a more fundamental view, Ghosh et al. (2009) demonstrated that rates of meiotic recombination in families with a DS child in India were essentially the same as in a U.S. population, pointing to a basic identicalness of nondisjunctional mechanism across these populations.

Other Aneuploidy

The figures for DS are of most interest, as this condition *(1)* produces a major mental handicap, *(2)* implies a major burden for parents in that survival well into adult life is now the norm, and *(3)* is the commonest single chromosome defect in newborns. But the data for other aneuploidies are important. Women seeking advice on their age-related risk and considering prenatal diagnosis should know also that some other rather uncommon trisomies of severe effect (13 and 18) might be detected; and that, on the other hand, there are some age-related sex chromosome aneuploidies (XXX, XXY) that have much milder, but not trivial, effects. Tables 24–4 and 24–5 set out age-related risk estimates for these other categories of aneuploidy. There is also the possibility, irrespective of maternal age, that some other type of chromosome defect might exist. Table 24–6 sets out the risk for any chromosomal defect, whether maternal-age associated or not, to be detected at prenatal diagnosis. To put these figures into some perspective, we remind the reader that the prevalence of unbalanced chromosomal abnormality in the whole newborn population is approximately 0.5%, or 1 in 200 (see Table 1–3 in Chapter 1). Another window of observation afforded in recent years is at preimplantation diagnosis, and increasing rates of aneuploidy seen in biopsied embryos, according to the mother's age, are demonstrated in Table 24–7.

Table 24–4. Maternal-Age-Specific Risks for Trisomies 13 and 18 Calculated at 10 Weeks Gestation (the Usual Yime for CVS), 16 Weeks (Amniocentesis), and at Live Birth

MATERNAL AGE*		TRISOMY 13				TRISOMY 18		
YEARS		10 WEEKS	16 WEEKS	LIVE BIRTH		10 WEEKS	16 WEEKS	LIVE BIRTH
20	1 in	6500	11000	14300	1 in	2000	3600	10000
25		5600	9800	12500		1750	3200	8300
30		3700	6500	11100		1200	2100	7200
35		1500	2600	5300		470	840	3600
36		1200	2000	4000		370	660	2700
37		900	1600	3100		280	510	2000
38		700	1200	2400		220	390	1500
39		530	920	1800		170	300	1000
40		400	700	1400		130	230	740
41		300	530	1200		95	170	530
42		230	400	970		70	130	400
43		170	300	840		55	95	310
44		130	220	750		40	70	250

* Age at the indicated gestation or at birth, respectively.

Source: Prenatal data from Tables 3 and 4 in Snijders et al. (1995), and modeled livebirth estimates from Appendix A in Savva et al. (2010). Figures are rounded.

Table 24–5. Maternal-Age-Specific Risks for 47,XXX and 47,XXY at Amniocentesis and at Live Birth

MATERNAL AGE	XXX			XXY		
	AMNIO	LIVE BIRTH		AMNIO	LIVE BIRTH	
YEARS	‰	‰	1 IN	‰	‰	1 IN
33		0.4	2500		0.4	2500
34		0.5	2000		0.4	2500
35	0.4	0.5	2000	0.5	0.6	1650
36	0.5	0.6	1650	0.6	0.7	1450
37	0.7	0.8	1250	0.8	0.9	1100
38	0.9	0.9	1100	1.1	1.1	900
39	1.1	1.1	900	1.4	1.4	700
40	1.4	1.3	770	1.8	1.7	600
41	1.8	1.6	630	2.4	2.2	450
42	2.2	1.9	530	3.1	2.7	370
43	2.8	2.2	450	4.1	3.4	300
44	3.6	2.7	370	5.4	4.3	230
45	4.5	3.2	310	7	5.4	180
46	5.7	3.8	260	9	6.8	150
47	7	4.5	220	12	8.5	120
48	9	5.5	180	15	11	95
49	11	6.5	150	20	13	75

Source: From data in Tables 20.4 and 20.7 in Hook (1992). Figures are rounded.



Table 24–6. Maternal-Age-Specific Risks for All Unbalanced Chromosomal Abnormalities at Chorionic Villus Sampling[a] and at Amniocentesis[b], for the Age Range 33–45 Years

MATERNAL AGE[c]	CHORIONIC VILLUS SAMPLING		AMNIOCENTESIS	
	%	1 IN	%	1 IN
33			0.5	200
34			0.6	160
35	0.9	115	0.8	120
36	1.2	85	1.0	100
37	1.5	65	1.2	80
38	2.0	50	1.5	65
39	2.5	40	2.0	50
40	3.5	30	2.5	40
41	4.5	22	3	33
42	6.0	17	4	25
43	7.5	13	5	20
44	10	10	6	17
45	13	8	7	14

[a]Including invariably lethal defects

[b]Including those for which there is no maternal age effect.

[c]Age at time of procedure.

Source: Taken from "averaging" data for ages 33–45 in Tables 20–7 and 20–8 (amniocentesis), and for ages 35–45 from Table 20–10 (CVS) in Hook (1992). Figures are rounded.

Table 24–7. Aneuploidy Rates in 591 Embryos Tested at Preimplantation Genetic Diagnosis in the Course of In Vitro Fertilization, with Respect to Chromosomes 13, 15, 16, 18, 21, 22, X and Y, According to Maternal Age

MATERNAL AGE (YEARS)	25–34	35–37	38–39	40–41	42–44
% Aneuploid	8	10	18	26	30
% Other abnormal	31	30	35	31	31
% Normal	61	60	47	43	39

Source: From Munné et al. (2002b).

No Parental Age Effect in Some Defects

There is no discernible increasing risk with increasing maternal age for the following chromosomal abnormalities: de novo rearrangement, XYY, triploidy, and unbalanced karyotype due to transmission of parental translocation. For monosomy X, the risk actually lessens with increasing maternal age. With no firmly proven paternal age associations, advanced paternal age is not of itself a particular indication for chromosomal prenatal diagnosis, although a case might hypothetically be made for a much older (sixties and older) father.

Secular Changes in Maternal Age Distribution and Down Syndrome Prevalence

Changing maternal age profiles in a population will influence the birth prevalence of DS. In the England of Shakespeare's time, few women lived long enough to bear children in older age, and along with the effects of poor survival in DS, perhaps no more than 100 individuals with trisomy 21 then existed in that country, in a total population of 4 million (Berg and Korossy, 2001); a similar situation exists in some developing countries today. (Nevertheless, Levitas and Reid, 2003, were able to record a number of probable and possible depictions in art from centuries past, and indeed Martinez-Frias, 2005, has presented a photograph of a terra-cotta head, made in about 500 CE in Mexico, that convincingly captures the essence of the DS facies.) In New Zealand in the 1920s, maternal mortality was much less of an issue but family planning was rudimentary, and about 45% of all mothers were aged 30 and over. The great majority (about 90%) of all DS babies from that period, at least those surviving to the 1960s to have a chromosome study, were born to mothers in this age group. Over the next four decades, contraceptive practices became gradually more widespread. By the late 1960s, most women were completing their families while still in their twenties, and "older mothers" made much less contribution to the overall birth rate. Only 20% of all mothers were 30 and over; and the proportion of all DS babies born to this age group had fallen to 53% (Gardner et al., 1973a). We presume, therefore, that the birth

prevalence of DS in New Zealand progressively fell over the period 1920–1970.

Hook (1992) reviewed the prevalences of DS in various parts of the world during the early 1980s, in relation to the proportions of mothers aged 35 and older. The former Czechoslovakia had the lowest proportion, 3.6%, of older mothers, and Northern Ireland, at 11.1%, the highest. As expected, the observed rates of DS births showed a relationship, with 1.06‰ (per thousand) in Czechoslovakia, and 1.60‰ in Northern Ireland. In the 1980s and 1990s, there was a reversal of the maternal age trend in several parts of the world, with older mothers closing the gap on their younger counterparts. In South Australia, for example, after falling to a trough around 1975–1978, the fraction of mothers over age 35 years progressively rose, and the birth prevalence of DS was anticipated to rise from a low point of about 0.9‰ in the late 1970s to greater than 1.5‰ in 1990–1994 (Staples et al., 1991). In Israel, maternal age dipped in 1978 to a low of 8% of Jewish mothers being 35 or older, and rose to 17% by 1992; and in Alberta, Canada, the comparable figures are 4% in 1980, to 16% in 2007 (Shohat et al., 1995; Lowry et al., 2009). These trends are similar in most affluent countries.

Trisomies 13 and 18 have a maternal age association, and so it is not surprising that similar changes in prevalence are observed. From U.K. and Australian data (and adjusting for prenatal diagnosis and termination), the live birth rates increased by 13% and 25% for the two trisomies, respectively, from 1989–1996 to 1997–2004 (Savva et al., 2010).

The DS birth prevalence is considerably influenced by the use of prenatal diagnosis and selective pregnancy termination, these options becoming widely available in many countries from the 1970s and 1980s. In England and Wales over the period 1974–1987, 14% of potential DS births were avoided by selective abortion, reducing the birth prevalence from 1.26‰ to 1.08‰ (Cuckle et al., 1991). The South Australian figures noted earlier are estimates of the birth prevalences had termination not been used; in fact, the actual prevalences are correspondingly less (Cheffins et al., 2000). More recent data have come from large studies from England and Wales (Morris and Alberman, 2009) and in the United States (Egan et al., 2011), and these reflect the increasing access to noninvasive screening. In England and Wales from 1989 to 2008, while the number of DS diagnoses overall rose very substantially, by 71%, concomitant with a changing maternal age profile, the number of actual DS births fell marginally, from 755 to 743. The proportion choosing termination over this period remained constant, at 92%. In the United States over the period 1989–2006, the reduction in DS births has varied according to region, with the observed births 44% of expectation in the West, compared with 68% in the Midwest; in the Northeast and the South the figures fell between, but tending more toward those of the West. From their analysis, Egan et al. (2011) conclude that “a Down syndrome fetus is more likely to be prenatally diagnosed and terminated in the West and least likely to be diagnosed and terminated in the Midwest.” The influence of termination is more noticeable among older women: in Alberta, Canada, in 2007, the birth rate in mothers in their forties was 13.2‰, but it would have been 21.5‰ had not termination been available, whereas the comparable rates for 20–24 year-olds were 0.55‰ and a not much greater 0.76‰ (Lowry et al., 2009).

Some centers offer a slightly different picture, with the shift to the right in the maternal age curve counteracting the influence of prenatal testing. In Switzerland, the mean maternal age rose from 26 years in 1980 to 30 years in 1996, and the incidence of DS remained practically unchanged (Mutter et al., 2002). Indeed, in Japan, where recourse to termination is less frequently sought, the birth incidence is rising, as the maternal age spectrum moves to the right (Takeuchi et al., 2008). Prevalence is also influenced by the greater survival of children with DS in recent decades. The survival figure to age 1 year for Western Australia rose from 83% in those born during 1966–1975, to 94% for the period 1991–1996, and survival to age 10 years to 85% (Leonard et al., 2000).

Screening for Fetal Trisomy

The birth of a child with Down syndrome is preventable, in that prenatal diagnosis (amniocentesis or chorionic villus sampling) is possible, and a known abnormal pregnancy can be terminated. The definitive prenatal diagnostic procedures of amniocentesis or CVS could not realistically be offered to the whole population of pregnant women,[2] but screening allows a subgroup at increased risk to be identified, who then can be offered definitive testing. “Screening” in this context should meet three criteria: it should identify women who are at increased risk, prior to their having a definitive diagnostic test; it should be offered systematically to pregnant women, who are considered only to be at population risk; and it should be seen as beneficial to those who receive it, either in terms of choosing termination, or of being prepared for the birth of a child with DS (Weisz and Rodeck, 2006).

The screening tools are the taking of a blood sample, and the performing of an ultrasonogram. Certain biochemical markers in the mother's serum may have altered concentrations, whether increased or decreased, if she is carrying a trisomic pregnancy; presumably, these differences reflect perturbation in the trisomic feto-placental unit. An assessment is made of the degree to which each level differs from expectation, and these data are factored into an algorithm that takes into account the prior risk due to maternal age (Spencer, 2007). Sophisticated computer packages are employed to calculate an overall risk figure. And, subtle differences in fetal morphology are detectable on ultrasonography.

The screening approach differs according to the timing in pregnancy, whether in the first or second trimester (or possibly both: “integrated screening”). If the calculated risk is greater than that of a certain threshold risk figure (usually taken as 1 in 250), the pregnancy is regarded as being at “increased risk,” and definitive testing is then offered. Since other aneuploidies can also influence the measured indices, the test procedure in practice becomes broader than just a trisomy 21 screen.

If and when the promise were to be realized of definitive prenatal diagnosis of trisomy by the analysis of free fetal DNA in the maternal blood (p. 423)—a form of noninvasive prenatal diagnosis (NIPND)—the screening approach would need to be reassessed. As Wright and Chitty (2009) comment, “It may ultimately be possible to replace the multistep process [of ultrasonography and serum biochemistry] with a single *diagnostic* blood test based on cell-free fetal DNA or RNA.”[3] Even so, it is to be borne in mind that the current screening format picks up other conditions, including chromosomal ones, besides the major trisomies; and thus we would anticipate that screening by maternal serum biomarkers and ultrasonography will have a place in pregnancy management for some time yet.

First-Trimester Ultrasonographic Screening

Ultrasonographic scanning is applied during the window of 11–14 weeks inclusive. This particular parameter is assessed: the degree to which the skin at the neck is separated from the underlying tissue by fluid. Since this fluid does not reflect the sound wave on the scan, it is referred to as “nuchal translucency”; “nuchal thickening” is another expression. An increased nuchal translucency is associated with DS.[4] Bekker et al. (2006) propose that the underlying cause is anomalous development of the lymphatic system in the region of the neck; and it appears that this development is susceptible to a chromosomal imbalance. Another ultrasonographic marker of DS is reduced length, or absence, of the fetal nasal bone; this may be less helpful in the first than in the second trimester (Collado et al., 2005; Ramos-Corpas et al., 2006).

First-Trimester Biochemical Screening

At present, the two first-trimester analytes most commonly measured are β-hCG and pregnancy-associated plasma protein-A (PAPP-A)[5], the former typically high and the latter low in a DS pregnancy. We may anticipate that other factors will come to be recognized, and which may improve sensitivity; one such, for example, may be placental growth factor (Cowans et al., 2010).

First-Trimester Combined Ultrasonography and Biochemical Screening

A better detection is achieved through a combination of first-trimester nuchal translucency assessment and the measurement of maternal serum β-hCG and PAPP-A. If the blood test is done first, these results can be held pending the ultrasound, and the combined figure can be available shortly after the scan is done. Detection rates are typically 80%–90%, for a false-positive rate of 5% or less (Spencer, 2007). The validity of this approach in more precisely targeting an increased risk population is attested in the experience from Denmark, where a national program was put in place in 2004. The number of diagnostic procedures (amniocentesis or CVS) declined from 7524 in 2000 to 3510 in 2006; and yet, over the same period, the number of newborns with Down syndrome fell from around 50 to 30 per year (Ekelund et al., 2008).

As the first trimester merges into the second at 12–13 weeks, testing started in one trimester can overlap with the next, and the sensitivity of PAPP-A can fall away. To

counteract this, Jaques et al. (2007) in Victoria, Australia, added in three other analytes (AFP, estriol, and inhibin-A), for those women having the blood sample drawn at 13 weeks, and they referred to this as “augmented screening.” They achieved a 91% detection rate, for a false-positive rate of 4%.

Second-Trimester Biochemical Screening

The analytes measured in many jurisdictions for second-trimester screening comprise α-fetoprotein (AFP), estriol, the β component of human chorionic gonadotrophin (β-hCG), and inhibin-A (four analytes: hence, the “quadruple test”[6]). In trisomy 21, the AFP is low, hCG high, uE3 low, and IA high. The proportions of trisomy 21 and other aneuploidies detected by this approach are approximately equal (Sheridan et al., 1997). About a quarter of women aged 37 and older will get an increased risk result, and the remainder a low risk result; as noted earlier, some in this latter group might then choose to forego amniocentesis.

Second-Trimester Ultrasonography

Several “soft signs” on second-trimester ultrasonographic fetal assessment point to an increased likelihood for DS. An advantage is that this procedure is often done routinely, as part of normal obstetric management, and thus a DS screen can be added on essentially at no additional cost. However, the observations do not lend themselves to a ready analysis in terms of adjusting the level of risk; further, the frequency of these “soft signs” in normal fetuses leads to a high false-positive rate. As in first-trimester screening, the length (or absence) of the fetal nasal bone is proposed as a useful adjunctive observation, this measurement being shorter in a trisomic 21 fetus. Ethnic differences in nasal anatomy need to be accounted for (Collado et al., 2005; Jung et al., 2007; Spencer 2007; Casasbuenas et al., 2009).

Integrated Screening

In theory, the best detection rate could be achieved from a combination of first- and second-trimester screening, as high as 94%, for a 5% false-positive rate, the result offered as a single report following the second-trimester testing (Wald et al., 1999). In practice, from a 3½ year study in Hong Kong, Lam et al. (2002) reported an 86% detection rate, which while less than 94% was a better figure than that achieved with either first- or second-trimester screening alone. A U.K. advisory group in 2008 came out against integrated screening as the preferred policy, and in favor of first trimester combined; but this drew a sharply critical response from Rodeck and Ogilvie (2009), who noted the advantages of a lesser rate of invasive testing; a greater use of a more widely available and more accurate procedure (that is, amniocentesis); and more opportunity for natural loss to supervene, with the integrated approach.

Okun et al. (2008) provide a useful practical assessment, from their experience in large populations at two major hospitals in Toronto, Canada, in which they could compare first-trimester screening (nuchal translucency plus serum PAPP-A and free ß-hCG) at one hospital, with integrated screening (first-trimester screen followed by second-trimester serum AFP, ß-hCG, and estriol) at the other. Both approaches gave satisfactory detection and false-positive rates, the integrated approach being (unsurprisingly) slightly superior (88.4% cf. 83.9% detection). These authors point to the need for those who do the nuchal ultrasonography to have “energy and enthusiasm,” in the setting of a busy prenatal clinic. They comment that, as women have become more sophisticated in their understanding of these differing approaches (the study was done over the period 2003–2005), they are well able to make a distinction, and “those most interested in early detection tend to choose first trimester screening, whereas women who wish to minimize the chance of a screen-positive result choose integrated screening.” It was the policy at both hospitals to refer women returning a nuchal translucency measurement of 3.5 mm or above for immediate genetic counseling, given that serum biochemistry, even if normal, is unlikely to bring the risk figure below the threshold for action (thus, a form of contingent screening; see later).

Contingent Screening

This is a variation on integrated screening, and an example of how this might be applied is given in Rozenberg et al. (2007). The first-trimester screen is offered, with the expectation of proceeding, in due course, to second-trimester testing. However, a “high-risk” result (taken as greater than 1 in 64) would allow immediate access to a CVS.[7] If the screen result were “low-risk” (1 in 1500 or less), no further testing is proposed. With an “intermediate” result (1 in 64–1500), where the majority will lie, the woman continues on track to second-trimester screening, with both results eventually integrated into a final estimation.

Twin Pregnancies

Two feto-placental units lead, it would be expected, to the production of twice as much of the particular biochemical substance that is then conveyed into the maternal bloodstream. Muller et al. (2003) examined the second-trimester and Spencer et al. (2008) the first-trimester analyte levels in cohorts of twin pregnancies; and the multiple of median (MoM) values were essentially double those of singleton pregnancies. The valid MoMs for risk evaluation can thus be derived by dividing the observed result by approximately 2. Intriguingly, monochorionic (and presumably monozygous) twins at first trimester have a somewhat lower PAPP-A mean (1.76 MoM) than do the dichorionic (2.25 MoM), but with the average (2.12 MoM) being close to double; if chorionicity is distinguishable at ultrasonography, adjustment can be made by applying the appropriate PAPP-A divisor.

A theoretical complicating factor, in the case of one (dizygous) twin being trisomic 21, is that the normal co-twin might “dilute out” the abnormal serum biochemistry, and thus invalidate the test result. However, in a large French study addressing a second-trimester population, such an effect, if present, was marginal (and not significant statistically), and screening in this setting was considered to be effective (Garchet-Beaudron et al., 2008).

Garchet-Beaudron et al. (2008) point out other issues relating to twin pregnancies. Logically, the age-related risk for DS should double in a dizygous twin pregnancy. But such logic appears not to apply: actual observation does not record such an increase. The technical procedures in the event of an increased-risk result are more demanding: double amniocentesis, with each sac sampled separately; and, if one twin is trisomic and selective termination is sought, the normal twin is placed at risk. Screening in twin pregnancies requires special expertise. In the case of a “vanishing twin” at the first trimester, as manifest by a second, empty sac, it may be prudent to confine the screening analysis to the nuchal translucency alone (Spencer et al., 2010).

In Vitro Fertilization Pregnancies Following Ovarian Stimulation

The biology of an in vitro fertilization (IVF) conception, following stimulated ovulation with the production of several oöcytes, is subtly different from the natural case. Simplistically, this may reflect the fact of an oöcyte having been released before it was fully prepared to do so; a firmer suggestion is that multiple simultaneous oöcytes generate multiple corpora lutea, which may influence the hormonal milieu, the corpus luteum being a source of hormones, including inhibin-A (Treetampinich et al., 2000). At first trimester, the inhibin-A and β-hCG levels may be marginally elevated, and PAPP-A reduced, compared with the levels from normal conception (the latter two alterations potentially edging up the false-positive rate), although not all studies are in agreement. In the second trimester, changes in levels are slight, but statistically significant: estriol lowered, and β-hCG and inhibin-A raised, but again there is not universal agreement (Lambert-Messerlian et al., 2006; Tul and Novak-Antolič, 2006; Weisz and Rodeck, 2006; Anckaert et al., 2008; Amor et al., 2009). The screening analytes appear not to be influenced when the artificial reproductive technology does not involve ovarian stimulation.

Couples achieving a pregnancy by IVF may find screening particularly attractive, since it involves no invasive procedure that might put at risk a pregnancy in which there has been so much “investment” (Meschede et al., 1998b). In assessing the degree of risk for aneuploidy, note that it is the age of the woman (whether of the pregnant woman herself, or a donor) at the time the ovum was collected that counts, not her current chronological age.

Interpretation of Screening Results

What do these various figures mean? A little epidemiology is in order. Imagine a group of 10,000 pregnant women, of all ages. Assuming a birth prevalence for DS of 1.2‰,

we can take it that 12 would otherwise give birth to a baby with DS. If the particular screening approach has a detection rate of, say, 85%, 10/12 of these DS pregnancies would be recognized as being at increased risk, and they could be identified at prenatal diagnosis. The remaining 15% who are carrying a DS fetus (2/12) would fail to be recognized. If the false-positive rate is, say, 4%, 400 women would have an increased-risk report following screening, but followed by a normal result from the amniocentesis or chorionic villus sampling. Putting these figures in the conventional format, we have:



	FETUS WITH DS	FETUS NOT WITH DS	TOTAL
Test shows “increased-risk”	10	400	410
Test shows “low-risk”	2	9588	9590
Total	12	9988	10000

The *detection rate* (sensitivity) of the test is 10/12 (~85%). Thus, 15% of women with a trisomic 21 fetus will be missed by the test. The *positive predictive value* of the test is only 10/410 (2.4%). Thus, 97.6% of women returning an “increased-risk” result will not have a DS baby. The *negative predictive value* is 9588/9590 (99.98%); in other words, a “low-risk” result means a 99.98% chance for an unaffected baby.

The false-positive rate is an important parameter: as noted earlier, this represents the fraction of women who go on to have an invasive definitive test, which then returns a normal chromosomal result. Clearly, the smaller this figure, the better. The trade-off is this: the smaller the false-positive rate, the less the detection rate. To judge the effectiveness and acceptability of the screening, we can declare a false-positive rate that is desirable, and this would then determine what the detection rate will be; or we can choose a preferred detection rate and accept the false-positive rate that this would incur. Weisz and Rodeck (2006) summarize as follows. A typically desired false-positive rate is 5%; based upon this, the detection rate is noted as DR_5. Desired detection rates of 85%, or 90%, would come at a cost of false-positive rates noted as FPR_{85} and FPR_{90}. The detection rates, and false-positive rates, that are thus determined, are set out in Table 24–8.

Table 24–8. Detection Rates, as Percentages, According to False-Positive Rates, and Vice Versa, due to Four Studies, as Applied to Second-Trimester Maternal Serum Screening

TEST	DR_5	FPR_{85}	POSITIVE PREDICTIVE VALUE
Triple test (S)	77	9	1 in 68
Triple test (F)	69	14	—
Quadruple test (S)	83	6	1 in 32
Quadruple test (F)	81	7	1 in 37

DR_5 = the detection rate that can be expected for a desired false-positive rate of 5%.

FPR_{85} = the false-positive rate that can be expected for a desired detection rate of 85%.

The triple test analytes are α-fetoprotein, estriol, and β-hCG; the quadruple test adds in inhibin-A.

S = data from the SURUSS study (Serum, Urine, and Ultrasound Screening Study); F = data from the FASTER study (First and Second Trimester Evaluation of Risks Trial).

Source: From Weisz and Rodeck (2006).

The Understanding of Women Who Have Screening

The interpretation of a maternal serum test result to the patient is fraught with potential for confusion. The major pitfall is that an “increased-risk” test result may sometimes be understood by the woman and her medical advisor to mean that the pregnancy is likely to be affected. As we showed earlier, *the great majority of women who screen “positive” will go on to have a normal baby*. Counselors doing this work need a clear awareness of these issues, so that they can enable their patients to understand, intuitively or explicitly, the concept and relevance of a low positive predictive value. The counselor is referred to Macintosh's (1994) essay “Perception of Risk” for a very readable and practical commentary upon these issues, and to Marteau and Dormandy (2001) for an overview of the complexity of the issues. The ideal is that those having a screening test for DS should have a basic awareness of the condition, and of the rationale of the screening procedure, and that their beliefs and perceptions and attitudes should be reasonably consonant with the aims and practice of the program.

The ideal is not necessarily met. Jaques et al. (2004), in a paper provocatively entitled “Do Women Know That Prenatal Testing Detects Fetuses with Down Syndrome?” surveyed responses from pregnant women 37 years and older in Victoria, Australia in 1998–1999; and the answer to their question was, disconcertingly, that “Down syndrome” was not mentioned as a reason for undergoing pregnancy testing in almost 40% of respondents. Not every woman will respond “rationally” to an increased-risk interpretation, according to the view of rationality as seen by the providers of the screening program. Those who enter into a screening program without being properly aware of the implications may find themselves “in an untenable situation—anxious about a positive result, but unwilling to incur the risks of diagnostic testing” (Kuppermann et al., 2006). Depressive symptoms, and thus a reduced capacity to make clear decisions, may be exacerbated in those with a predisposition, and Hippman et al. (2009) see a role for the counselor in recognizing this. The report from Toronto of more recent date (Okun et al., 2008), in which the program was well received, is rather more encouraging; and equally a subsequent (2003–2004) study from Victoria (Jaques et al., 2010) suggested that women were responding objectively to risk figures (71% of women chose definitive prenatal diagnosis after an increased-risk screen result, versus only 2.5% after a low-risk result). For those who are better informed, understanding is by no means a neutral matter, and Williams et al. (2005) refer to the role of women as “moral pioneers,” in coming to terms with the ethical issues that readily available screening may, in these modern times, present. Susanne et al. (2006) assessed responses in women who had had what turned out to be a false-positive screening result, following them prospectively through the pregnancy and after the baby was born. Several declared that they had “withheld” their pregnancy, and only returned to reacceptance after the normal chromosomal result from amniocentesis had been conveyed; nevertheless, most would have the same testing in a future pregnancy. Counselors need to be well attuned

to these several complexities; and if a woman's family physician can share in the decision-making process, this is typically well received (Légaré et al., 2011).

Concerning the facts about DS itself in the context of pregnancy screening, leaflets are the simplest means of conveying information, and many clinics/jurisdictions produce their own material, the quality of which may vary considerably (Murray et al., 2001). Videotapes may be helpful (Hewison et al., 2001). It is a fine matter to judge what should be the level and tone of the information. Bryant et al. (2001) reviewed the leaflets produced in a number of clinics in the United Kingdom and considered that the viewpoints expressed were, in the main, weighted unduly negatively toward DS. It is true that information ought to be couched in such terms that it will be useful, in the fullest sense of that word, to the wide range of people for whom it is intended (and see p. [link]). Equally, the comment can be made that attempting to neutralize negative aspects of DS may send a mixed message, since being given the option of abortion in order to avoid having a DS child rather plainly implies that having such a child may not be a desirable outcome. The view that is offered should be clear, accurate, and even-handed.[8]

Effect of Screening upon Prevalence of Down Syndrome

In several centers, the increasing acceptance of maternal serum screening has been associated, as expected, with a reduction in the number of DS babies being born, above that achieved by maternal age-targeted amniocentesis. Cheffins et al. (2000) assessed the situation in South Australia. The fraction of pregnant women having screening rose from 17% at the time of its introduction in 1991 to 76% by 1996; in consequence, the proportion having amniocentesis rose from 6% to 10%, with younger mothers the main contributor to this rise. The resulting increase in the detection of fetal trisomy 21, and with termination of the affected pregnancies, caused the birth prevalence of DS to fall by more than half—by 60%, to be precise—from 1.05 to 0.42 per 1000 births. This fall took place in spite of a "natural" increase in prevalence due to the mothers in 1996 being older. A very similar picture is seen in Belgium (Verloes et al., 2001). During 1984–1989, 244 cases of trisomy 21 were diagnosed in the Genetic Centers of Liège and Loverval, 17% of these at prenatal diagnosis. A decade later (1993–1998), of the 294 diagnoses of trisomy 21, the fraction detected prenatally had risen to 56%, and over 90% of these pregnancies had been terminated. Theoretically (and very probably actually), this reduced the birth prevalence from 1.26 to 0.62 per 1000, a fall of just over a half. More broadly, the pickup rate at prenatal diagnosis for all aneuploidies has increased, due to the identification of increased risk pregnancies afforded by these screening programs.

If screening is not widely offered, an inappropriate section of the population may be targeted for prenatal diagnosis, and the reduction that is achieved in DS incidence will be less. In Denmark over the period 1980–1998, there was a rather high level of CVS and amniocentesis procedures being done, but mostly in lower risk pregnancies, and this yielded about a 30%–40% reduction in the expected DS incidence. This figure could have been higher, for a lesser number of invasive procedures, had screening allowed a preselection of women at increased risk (Larsen et al., 2001).

Previous Pregnancy with False-Positive Result

If there has been a false-positive test result from second-trimester maternal serum screening in one pregnancy, there is a considerable likelihood, about 20%, that the same thing could happen in a subsequent pregnancy. Naturally, maternal factors influence the process whereby analytes produced by the fetus and placenta are transferred to the maternal bloodstream, and it is unsurprising that these same factors might obtain in sequential pregnancies. This phenomenon can readily be taken into account in a following pregnancy, by applying to the observed MoM, a divisor derived from the MoM values that had been seen in the initial pregnancy (Wald et al., 2004).

Other Abnormalities

Trisomies 13 and 18, Rare Trisomies, Other Aneuploid States.

While the prime focus of screening is on trisomy 21, a side benefit is the detection of other, and typically more severe chromosomal disorders. Trisomy 13 and trisomy 18 both show reduced levels of β-hCG and PAPP-A at first-trimester screening, more so in trisomy 18, along with increased nuchal translucency or frank cystic hygroma; and using a trisomy 21 analytical algorithm, about three-quarters of these trisomies can be detected, for a false-positive rate of 3%–6% (Breathnach et al., 2007; Kagan et al., 2008b). If specific algorithms for trisomies 13 and 18 are applied, and if an assessment of fetal heart rate is factored in, detection reaches 95%. Few other trisomic pregnancies proceed through to the time of screening. Unsurprisingly, those that do, display abnormalities at screening. For example, in trisomy 22 at first-trimester screening, the β-hCG is very elevated, PAPP-A somewhat reduced, and fetal growth restriction is typical (Sifakis et al., 2008). In nonmosaic trisomy 9, the biochemistry is similar to that of trisomy 18 (Priola et al., 2007), and the same may apply in the mosaic case. An increased nuchal translucency can be associated with a number of rare aneuploid states, partial or complete (Chen et al., 2005a). For example, β-hCG can be very high in fetal cri du chat syndrome (Torun et al., 2009), and similarly in confined placental mosaic trisomy 16, in which AFP is also elevated and PAPP-A low (Neiswanger et al., 2006; Petracchi et al., 2009). The 22q11 deletion syndrome, of which a heart defect is a frequent component, might have seemed a useful candidate for testing in the context of an increased nuchal fold; but in a smallish study of 146 pregnancies with a nuchal translucency above the 99th centile and otherwise normal, Lautrup et al. (2008) identified none with this deletion. At secondtrimester quadruple-test screening, about two-thirds of non-DS aneuploidies are detected, for a falsepositive rate of 9% (Breathnach et al., 2007). The fact remains, however, that about half of all fetal chromosomal abnormalities, and which would lead to substantial phenotypic defect, are not detected by these noninvasive tests (Grati et al., 2010).

Triploidy.

The biochemical indices at first-trimester screening in a triploid pregnancy are quite abnormal, and very differently so according to the category of triploidy, digynic, or diandric (p. 287). In the former, β-hCG and PAPP-A are both much reduced, while in the diandric type, β-hCG is greatly elevated, and PAPP-A marginally reduced. Likewise, ultrasonography is distinctly different, with severe growth restriction in the digynic type, and nearer normal growth but with an enlarged and partially molar placenta in diandric triploidy (Kagan et al., 2008a).

Notes:

[1] But note the startling suggestion from Hultén et al. (2008) that maternal gonadal mosaicism may be the important factor; see p. [link].

[2] Although the American College of Obstetrics and Gynecology (2007) has advocated that all pregnant women receive genetic counseling and are offered invasive testing. More realistically, screening is becoming widely available, and this College and the American College Medical Genetics recommend that screening be offered to all pregnant women (Skotko et al., 2009).

[3] De Jong et al. (2010) review ethical issues raised by this and other "easy, safe, and early" prenatal diagnostic tests.

[4] The sign is not specific for DS, and other chromosomal conditions may also display nuchal translucency. In one very specific example, a subtelomeric deletion of 5q, haplo-insufficiency of a gene *FLT4*, which is the basis of one type of lymphedema, has been proposed as the cause of the nuchal swelling (Rauch et al., 2003). If the karyotype is normal, there remains a small residual risk for some other type of fetal abnormality, most usually a cardiac defect. The risk is greater for larger nuchal translucency measurements; and the risk diminishes if subsequent mid-trimester fetal ultrasonographic anatomy is normal (Axt-Fliedner et al., 2009).

[5] PAPP-A is produced by the syncytiocytotrophoblast, the development of which is impaired in a trisomic pregnancy. The syncytiocytotrophoblast is anatomically close to the maternal uterine vascular circulation, and also to the celomic cavity; the same applies to ADAM12, a more recently developed first- and second-trimester marker (Wang et al., 2010b). Thus, these analytes have value as maternal serum markers (and also in celomic fluid analysis); but due to the barrier imposed by the amniotic membrane, they cannot be applied to amniotic fluid (Makrydimas et al., 2006).

[6] The "double test" uses AFP and estriol; the "triple test" is AFP, estriol, and β-hCG

[7] This approach is standard practice in some U.S. centers. If the ultrasound (nuchal translucency, nasal bone) reaches a certain threshold, CVS is offered then and there, while the woman is still on the examination couch. Otherwise, maternal serum testing is subsequently provided.

[8] These matters are dealt with in considerable detail in a document from the National Health Service of the United Kingdom, “Psychological Aspects of Genetic Screening of Pregnant Women and Newborns: A Systematic Review” (Green et al., 2004).











Chromosome Abnormalities and Genetic Counseling (4 ed.)

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Prenatal Diagnostic Procedures

Chapter: Prenatal Diagnostic Procedures
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THE MEANS to diagnose the fetal karyotype have provided medical cytogenetics with one of its major areas of application. The discovery of an abnormality allows the option of termination of the pregnancy or, later in gestation, a more suitable obstetric management. The main indications for prenatal cytogenetic diagnosis are the following: (*1*) the pregnant woman being of advanced childbearing age, (*2*) parental heterozygosity for a chromosome rearrangement, (*3*) the birth of a previous child with a chromosome defect, (*4*) abnormal maternal blood biochemistry, and (*5*) fetal anomaly detected on ultrasonography.

Fetal Ultrasonographic Anomalies

Mid-trimester ultrasonographic screening is a routine part of obstetric management, and the identification of a fetal malformation is a common indication for a chromosome study. In Victoria, Australia, for example, 20% of prenatal chromosome tests in 1999 were done on the grounds of ultrasound findings of a fetal malformation, or of a marker of aneuploidy (Webley and Halliday 2001). Certain major ultrasonographic defects are fairly specific: for example, holoprosencephaly predicts the likelihood of trisomy 13, fetal hydrops/cystic hygroma predicts monosomy X or trisomy 21, an endocardial cushion defect or duodenal atresia predicts trisomy 21, and conotruncal defects are associated with the 22q11.2 deletion. Certain renal defects have a frequent association with fetal aneuploidy, as do cardiac malformations generally (Amor et al., 2003; Wimalasundera and Gardiner, 2004; Carbone et al., 2011). Up to 33% of heart defects are associated with fetal aneuploidy, although in most there will be additional anomalies. Cysts of the choroid plexus (tissue within the cerebral ventricles) are a “soft marker” for trisomy 18, but not trisomy 21 (Walkinshaw, 2000); they are harmless in normal children (DiPietro et al., 2011).

On the specific question of rare autosomal abnormalities (rare trisomies, deletions, duplications, supernumerary markers, various other structural rearrangements), a large European series based upon reports from malformation registers in several jurisdictions linked ultrasound findings to cytogenetic results (Baena et al., 2003). Nearly half of all rare autosomal abnormalities showed fetal anomalies on ultrasonography, with heart and brain defects and growth retardation more often seen with deletions, and cystic hygroma, hydrops, and nuchal translucency more typically associated with trisomies and duplications. These rare abnormalities comprised 7% of all chromosomally abnormal prenatal diagnoses. A specific observation, such as aortic narrowing or a conotruncal defect, may allow a targeted cytogenetic study, such as FISH for the 7q11.23 deletion of Williams syndrome, and the 22q11 deletion (Krzeminska et al., 2009). Asymmetrical growth (head circumference vs. crown-rump length) may point to triploidy (Salomon et al., 2005). The acardiac fetus is often due to an otherwise unsurvivable autosomal trisomy, possibly tempered by mosaicism with a normal cell line, but their existence being maintained by a (karyotypically normal) monozygous co-twin (the “pump twin”).

In which cases should a chromosome analysis be conducted, following the discovery of structural anomalies by ultrasound examination? Staebler et al. (2005) examined the karyotypes (on classical cytogenetics) in 428 fetuses with ultrasound-detected anomalies over a 10-year period. The karyotype was abnormal in 9% of cases with an isolated malformation, and in 19% of cases with multiple malformations. The following isolated defects were typically associated with a normal karyotype: hydronephrosis with high obstruction, unilateral multicystic dysplastic kidney, gastroschisis, intestinal dilatation, cystic adenomatoid malformation, pulmonary sequestration, tumor, and vertebral anomaly. Thus, one of these as a single malformation is not an indication, whereas, clearly enough, the presence of multiple malformations would warrant chromosome study. Applying the more precise tool of array-CGH, Coppinger et al. (2009) and Kleeman et al. (2009) found that 2%–4% of pregnancies with abnormal ultrasound findings and a normal karyotype have a clinically relevant DNA copy number alteration. Although the number of cases was too small to stratify the data based on malformation type, these results suggest that all fetuses with ultrasound abnormalities might warrant a microarray analysis; and the American College of Obstetricians and Gynecologists (2009) has recommended that targeted microarray analysis be offered as an adjunct in prenatal cases with structural anomalies and a normal karyotype.

The minor marker of “increased nuchal fold thickness” (actually, this separation of the skin from the underlying tissue can extend from as far as the occiput down to the lower back) indicates an increased risk for fetal aneuploidy, the level of risk proportional to the degree of separation, and this observation is sufficiently robust that is used for pregnancy screening of aneuploidy, and especially during the first trimester (Chapter 24). This is particularly sensitive in trisomy 18, with the great majority, 90%, manifesting this sign in the first trimester (Sepulveda et al., 2010). Daniel et al. (2003a) reviewed 1800 cases in which an anomaly (an actual malformation, or a minor marker of aneuploidy) had been detected at ultrasonography, and assembled a table of risks of aneuploidy according to the findings (Table 25–1). The abnormal karyotypes included trisomies 13, 18, 21, triploidy, 45,X and mosaics, various autosomal and gonosomal duplications and deletions, rare trisomies, and de novo apparently balanced rearrangements. Souka et al. (2005) have assessed risks in the setting of the specific finding of an increased nuchal translucency, related to the degree of separation (Table 25–2). A practical question is this: if, following the observation of an increased nuchal translucency a CVS or amniocentesis is done, and the chromosomes are normal, is there a residual risk for some other type of fetal abnormality? If the translucency resolves, and if no defects (with particular focus on the fetal heart) are seen at 14–16 weeks gestation, the prognosis is good, with a better than 95% chance of a baby with no major abnormalities; and if a further scan at 20–22 weeks is normal, the risk is likely to be no different than the background population figure. If, on the other hand, the increased translucency persists, a possibility exists of a congenital infection or a genetic syndrome.

Table 25–1. The Likelihood of Discovering a Chromosome Abnormality at Prenatal Diagnosis, According to the Pattern of Defects Identified at Fetal Ultrasonography, for All Maternal Ages

DEFECTS	LIKELIHOOD OF AN ANEUPLOIDY
CNS/cranial shape plus cardiac*	53%
Key malformation,** singly or in combination	37%
CNS ± other***	21%
Nuchal translucency, first trimester ± other abnormality	25%
Nuchal translucency, second trimester ± other abnormality	13%
Cardiac ± other abnormality	9%
Pyelectasis/two vessel cord/echogenic bowel/short femur	6%
Other (singly or in combination)	3%

Notes: Some percentages are considerably higher or lower for older and younger maternal ages, respectively. These data were obtained prospectively.

* Excluding anencephaly/spina bifida.

** Cystic hygroma/hydrops/exomphalos/severe IUGR/duodenal atresia/talipes.

*** Excluding anencephaly/spina bifida/cardiac, including choroid plexus cysts.

CNS, central nervous system; IUGR: intra-uterine growth retardation.

Source: From Daniel et al. (2003a).

Table 25–2. Likelihood of Chromosome Abnormality, and of Other Outcomes, in the Setting of Increased Nuchal Translucency

NUCHAL TRANSLUCENCY	CHROMOSOME ABNORMALITY	MISCARRIAGE, FETAL DEATH	MAJOR FETAL ABNORMALITIES	ALIVE AND WELL
<95th centile	0.2%	1.3%	1.6%	97%
95–99th centile	3.7%	1.3%	2.5%	93%
3.5–4.4 mm	21%	2.7%	10%	70%
4.5–5.4 mm	33%	3.4%	19%	50%
5.5–6.4 mm	51%	10%	24%	30%
>6.5 mm	65%	19%	46%	15%

Note: The row “<95th centile” describes the baseline population risks.

Source: From Souka et al. (2005).

There are some subtleties in the choice of language when fetal anomalies are uncovered by ultrasound, as de Crespigny et al. (1996, 1999) discuss. We speak of the pregnant woman as a mother, yet she is not; neither is her husband/partner as yet a father. Equally, the fetus is not a baby, not acquiring that status until ex utero existence is achieved. But of course many parents-to-be, not to mention professionals (including us), use these words. Counselors should be sensitive to these subtleties. De Crespigny observes that, if an ultrasonologist should discover a fetal defect, using the terms “baby” and “mother” may exert indirect pressure on the couple to continue the pregnancy: “Although many women regard a fetus as a baby from the very beginning, others will be affronted if their doctor does not seem to recognize this difference between a fetus and a baby, which they may interpret as interfering with the pregnant woman's reproductive freedom.” As always, counselors will need to know their patients, and to judge the right words to use, and the way to say them (Benkendorf et al., 2001).

Twins

In the event of a twin pregnancy having been shown on ultrasonography, the question arises of an appropriate prenatal diagnostic procedure, if this is considered appropriate. A point to make here is that, while monozygous (MZ) twins would be expected to have the same karyotype, this does not invariably apply. Furthermore, the ability to interpret monozygosity is not perfect; and those MZ twins in which the split occurred shortly after conception, prior to the differentiation of the extrafetal tissues, may have the same ultrasound morphology of membranes (amnion and chorion) as would a dizygous pair. Thus, the advice is that dual amniocenteses, rather than CVS, may be the procedure of choice; and more especially so in the setting of discordance for an anatomical anomaly (Lewi et al., 2006).

Prenatal Laboratory Diagnostic Procedures

Since the early 1970s, prenatal diagnosis (PND) of chromosome disorders has been done by culture of amniotic fluid cells obtained by amniocentesis at about 16 weeks of pregnancy. A number of other approaches to PND have since been developed, ranging from preimplantation genetic diagnosis (following in vitro fertilization), through chorion villus sampling (CVS), to fetal blood sampling, and some more experimental procedures. Naturally, parents-to-be are anxious to have results as early as possible. A desire for

an early result needs to be balanced against a number of considerations which can include complexity of the procedure, both clinically and in the laboratory, procedural trauma and risks, reliability of results, cost, and the prior risk for a fetal abnormality. A useful source for the layperson is *Prenatal Testing: Making Choices in Pregnancy* (De Crespigny et al., 1998).

Molecular Methodologies

Four particular analytical procedures have enabled a much faster return of results for common aneuploidies: FISH, QF-PCR, MLPA, and higher resolution detection of any chromosome abnormality by microarray analysis (and see also Chapter 2).

Fluorescence in Situ Hybridization

Fluorescence in situ hybridization (FISH) using multiple colored probes and targeting the chromosomes most prone to survivable aneuploidy (13, 18, 21, X, and Y) bypasses the need for culture, whether the cells are from amniotic fluid, CVS, or fetal blood, and the result can be given within the space of one working day (Klinger et al., 1992; Morris et al., 1999; Weremowicz et al., 2001; Lim et al., 2002; Baart et al., 2004). This approach is particularly useful where the need for a rapid result is more pressing, such as when fetal anomalies have been discovered at ultrasound. In one small series with particular reference to the third trimester, Aviram-Goldring et al. (1999) showed an aneuploidy in 23% of pregnancies with intrauterine growth retardation and structural abnormalities: five with trisomy 21, and two with trisomy 18. Feldman et al. (2000) similarly applied amniotic fluid cell FISH to high-risk pregnancies (that is, with ultrasonographic abnormalities). They detected fourteen cases of trisomy 21, ten of trisomy 18, three of trisomy 13, four of monosomy X, and one triploid, in 4193 samples over the period 1996–1998, for a total abnormality rate of 11%. A limitation is that only those specific aneuploidies being tested for (the major autosomal trisomies and sex chromosome aneuploidies) are detected, and it is fair to say that conventional karyotyping is not about to be replaced by FISH (Morris et al., 1999). Waters and Waters (1999) comment that, with shortening times for standard karyotyping, there may actually be less pressure for a rapid result. While there is an attraction of being able to offer a next-day result, albeit a preliminary one, it is nevertheless worth noting that a benefit in terms of reducing anxiety and stress is only short term, and otherwise there appears to be little advantage (Boormans et al., 2010).

The question arises of false-negative results. Weremowicz et al. (2001) reviewed their experience over 1992–2000, during which time they applied FISH (using probes for 13, 18, 21, X and Y) to some 8% of the 11,000 amniocentesis samples coming into their laboratory for routine karyotyping, this 8% including cases with an increased risk (abnormalities on ultrasound, serum screen result). In the whole material, of 89 potentially detectable abnormalities, 75 (84%) were found. The missed cases included 8 with an inconclusive result, one with no result, and—more importantly—5 false-negatives. Of these latter 5, the true karyotypes were trisomy 18 (2 cases) and trisomy 21 (3 cases). Technical problems related to poor hybridization efficiency (low copy number of the DNA repeats being probed, for example), and maternal blood contamination of the fluid sample, are plausible explanations.

As have others, Weremowicz et al. note the usefulness of the FISH approach in being able to provide a rapid answer particularly when there are grounds for suspecting an abnormality, or if the pregnancy is more advanced, but they also emphasize the need for careful counseling so that patients are aware of the limitations. With respect to trisomy 21, Witters et al. (2002) had an encouraging record: in a similar study comprising 5049 amniotic fluid samples, in which interphase FISH was applied in parallel with conventional karyotyping, all 70 cases of trisomy 21 were detected, and no false-positive result arose. One false-positive is on record, however, probably due to technical aspects of probe hybridization (George et al., 2003). On the question of mosaicism, Van Opstal et al. (2001) note that FISH on uncultured cells may provide a more accurate picture than on cultured cells, the latter possibly being subject to selective pressure in vitro and the abnormal cells more prone to fail in culture. On the other hand, the class of amniocyte that grows preferentially in culture (namely, amniotic mesoderm) might, according to the reinterpretation of Robinson et al. (2002), more closely reflect the true embryonic state.

Focused FISH can be applied in specific circumstances. The ultrasound discovery of a cardiac outflow tract abnormality would, for example, point to the need for 22q11 analysis. A rapid diagnosis is particularly to be desired in the setting of parental heterozygosity for a chromosome rearrangement in which there may be a high risk for abnormality, and FISH can provide this. Thus, Cotter and Musci (2001) used subtelomeric probes for 5pter, 5qter, and 14qter to enable rapid diagnosis for a pregnant woman with the karyotype 46,XX,t(5;14)(p14.2; p13), she having had a previous child with cri du chat syndrome. Similarly, Pettenati et al. (2002) applied this approach in the setting of parental heterozygosity for a number of reciprocal and Robertsonian translocations.

FISH tests only that segment of the chromosome to which the probe binds. Inferentially, the complete chromosome is present; but this is not necessarily so. In the case of chromosome 18, it is the centromere which the FISH probe recognizes. We have seen a case in which amniocentesis was done on the basis of a maternal age of 40 (albeit that the Down syndrome risk on second-trimester maternal serum screening had been reduced to 1 in 164), and the figure for trisomy 18 was very low (1/8030). FISH showed three chromosome 18 signals. Fetal growth and morphology on ultrasonography were normal. The couple considered whether they might request termination but wanted to await the result of karyotyping. This showed a supernumerary minute marker—barely a speck—which appeared to comprise only 18 centromere: the karyotype was 47,XX,+mar.ish der(18)(D18Z1+)de novo[13]/46,XX[4] (case of M. Pertile). The pregnancy continued. The child subsequently born was 3 years old at most recent contact, and while she manifested a familial shortness, her cognitive and personality development were entirely normal (S. Fawcett, personal communication, 2010).

Quantitative Fluorescence Polymerase Chain Reaction

Quantitative fluorescence polymerase chain reaction (QF-PCR) relies on the use of molecular markers that display a high level of heterozygosity, such that the presence of three alleles—that is, a trisomy—can reliably be detected. This DNA-based test may enable rapid diagnosis. Pertl et al. (1999) performed the preliminary studies using QF-PCR with chorionic villus samples as the test material. The sequences assessed are chromosome-specific polymorphic small tandem repeats. There being three allelic forms of a particular marker allows the assumption of trisomy for that chromosome. There may need to be some choice of markers according to population: for example, Putzova et al. (2008) have determined a robust set of markers on chromosomes 13, 18, 21, and also for the X and Y chromosomes that are most informative in a population of Czech ancestry. In the experience of their laboratory, they could report a result within one working day, for 99.5% of samples (amniocytes or chorionic villi). This methodology detects only these major aneuploidies. From a large prospective study (4,176 amniocenteses), Speevak et al. (2011) drew the conclusion that QF-PCR may be suitable for pregnancies judged at risk only for a common aneuploidy, but where the risk is deemed to be higher, G-banding would be needed to detect the small residuum of significant abnormalities missed by QF-PCR.

Multiplex Ligation-Dependent Probe Analysis

Multiplex ligation-dependent probe analysis (MLPA) is a PCR-based assay that can combine probes to many chromosomal loci (Schouten et al., 2002). Its main application has been in the rapid detection of aneuploidy in prenatal testing (Shaffer and Bui, 2007). In a comparison with standard chromosome analysis of 1000 amniotic fluid specimens, MLPA had 100% sensitivity and specificity for identifying common aneuploidies (Kooper et al., 2008). A targeted application enables the diagnosis of microdeletion syndromes that would otherwise have escaped detection (Konialis et al., 2011).

Microarray Analysis

Microarray analysis either targeting specific regions (e.g., known microdeletion syndromes, subtelomeric regions, theoretical malformation pathways), or across the whole genome, has the potential to offer the widest prenatal diagnostic capacity, and usually within a few days turnaround time (South and Lamb, 2009). Retrospective studies have been helpful in demonstrating that microarray analysis will identify chromosome imbalances in prenatal samples with previously known abnormal karyotypes, or in samples from pregnancies with multiple fetal anomalies (Le Caignec et al., 2005; Rickman et al., 2006). Prospective studies demonstrate the additional benefit in the detection of imbalances either missed with banding, or at a resolution too small to be seen through the light microscope (Sahoo et al., 2006, Shaffer et al., 2008; Coppinger et al., 2009a; Van den Veyver et al., 2009).

Prenatal Diagnostic Clinical Procedures

Blastomere and Polar Body Biopsy

The techniques of preimplantation genetic diagnosis have advanced considerably in recent years, and a separate chapter (Chapter 26) is devoted to a treatment of this category of "prenatal" diagnosis.

Chorionic Villus Sampling

Chorionic villus sampling (CVS) is typically a first-trimester procedure, the usual time being at 10–11 weeks gestation. (The expression "placental biopsy" could also be applied, although in practice this term is used when the testing is done in later pregnancy; see below). The earlier period of diagnosis permitted by CVS may be seen as more useful in the setting of a higher genetic risk. If a genetic abnormality is identified, and abortion is chosen, this can be, prior to 14 weeks, a more private matter, and the termination procedure is an operative intervention (curettage or suction evacuation of the uterus). Couples are more likely to make a choice for abortion when the diagnosis has been made in the first trimester (Verp et al., 1988). There is potential in CVS for diagnostic difficulty due to the occasional detection of confined placental mosaicism (which may, for some chromosomes, carry a risk also for uniparental disomy). Nonmosaic results for the common aneuploidies are, however, highly reliable (Smith et al., 1999). In experienced hands there is a high degree of safety (Brambati et al., 2002; Brun et al., 2003), and indeed in one study in which lethal fetal diagnoses were accounted for, the loss rate was only 0.23% (1/436) (Nanal et al., 2003). However, there is some preliminary evidence that, at least for nulliparous women, the procedure may carry an increased risk for preeclampsia (Grobman et al., 2009).

Direct, Short-Term, and Long-Term Chorionic Villus Sampling.

Chorionic villi can be analyzed directly (same day), after short-term culture (next day or two), or after long-term (a week or so) culture; but most laboratories now offer only long-term CVS. Trophoblast is the source of the cell population studied at direct and short-term CVS culture. These cells are no longer extant (if they have not already been removed by trypsinization at sample receipt) after the first few days, and it is the mesenchymal core of the villus that provides the cells that are analyzed at long-term culture (and see Color Fig. 27–1).

> In the early 1990s there were disconcerting reports of an increased incidence of transverse limb deficiencies and tongue and jaw defects—"oromandibular-limb hypogenesis"—following early CVS (before 10 weeks, and especially up to 8 weeks). The association appeared likely to be causal, and one line of circumstantial evidence was that the rate of anomalies fell with increasing gestational stage from 9 to 11 weeks (Firth, 1997). Various mechanisms were proposed: oligohydramnios, bradycardia, hypovolemia, thromboembolism, vasoconstriction, antibody-mediated reaction, and increased apoptosis following disruption of end arteries (Luijsterburg et al., 1997). Given these observations, it became normal practice that CVS is not done earlier than 10 weeks.

Standard Amniocentesis

Transabdominal amniocentesis, at about 15–17 weeks gestation, with culture of cells for chromosome analysis, has been the standard cytogenetic prenatal diagnostic procedure for over a quarter of a century. It has a high degree of safety to both mother and fetus: maternal complications, or fetal injury due to direct trauma, are practically unknown. The risk for maternal Rhesus immunization (Rh-negative mother, Rh-positive fetus) can be circumvented by administering an antibody injection. The only significant complication is a procedure-related fetal loss rate of about 0.5%. The cytogenetic results are highly reliable. The biological sources of error are, first, that maternal rather than fetal cells, or a mixture of both, are sampled. In practical terms, this rarely causes a problem. Second, fetal mosaicism may go undetected, since only a limited number of cells can feasibly be examined. Very few examples of this error are recorded.

Amniotic fluid culture has a high success rate. Persutte and Lenke (1995) suggested that if amniotic cells fail to grow, for no obvious reason, there may be a substantial risk for fetal aneuploidy (13% of 32 cases in their preliminary study). This assessment was supported in a large systematic study from London (Reid et al., 1996), in which 42 failures (1%) among 4134 amniocenteses were followed up. Complete information could be obtained on all but one of these 42 cases. Karyotyping was ultimately done in most (78%) of these failed cases, and of these 19% revealed an abnormality (comparing with a 4% abnormality rate in the whole material). The clear lesson from these studies is that women having had a failed amniocentesis culture should be offered careful review and retesting.

The obvious disadvantage of standard amniocentesis with cell culture is that the results are not available until about 16–18 weeks. If the reason for the amniocentesis had been an "increased-risk" result from maternal serum screening, the procedure may not be done until 17–18 weeks, aggravating this difficulty. If a result cannot be available by 20 weeks, another procedure (placental biopsy, interphase FISH) may be worth considering. The outlook for the long-term health of the child does not differ between CVS and amniocentesis (Schaap et al., 2002).

Early Amniocentesis

In the late 1980s early (10–13 weeks) amniocentesis was proposed as an alternative to CVS. In a carefully controlled comparison, Nicolaides et al. (1994) found a 2%–3% additional fetal loss rate in early amniocentesis and, possibly, a higher incidence of talipes among subsequently born children. Daniel et al. (1998), comparing 10–14 week procedures with 15 weeks and upward, observed that the early amniocentesis samples were not quite as satisfactory, multiple needle insertions were more often required, and the pregnancy loss rate was greater. On the whole, the differences were not great, other than the loss rate of 2.2% in the early group compared with only 0.6% in the midtrimester group. Similar figures are reported in Collins et al. (1998). In the Canadian Early and Midtrimester Amniocentesis Trial, the findings for 11wk 0d through to 13wk 6d were somewhat more disconcerting, with more complications, and a higher culture failure rate (Delisle and Wilson, 1999). The procedure is rarely undertaken now.

Fetal Blood Sampling

Fetal blood is aspirated by direct puncture of a blood vessel, usually in the umbilical cord (cordocentesis). Before FISH analysis of uncultured cells (see above) came to be more widely used, cordocentesis was useful when speed of diagnosis was of the essence, in the setting of the detection of a fetal anomaly at ~18-week ultrasonography. The procedure once had a role in assisting resolution of mosaicism in amniotic fluid culture (Shalev et al., 1994), but this has largely been replaced by the use of FISH.

Placental Biopsy

In principle, this is the same as first-trimester CVS. The placenta is sampled by a transabdominal approach, and this is a straightforward procedure. The main application had been when a rapid result was needed, although newer methodologies (see above) have now largely by-passed that imperative. An insufficient amount of amniotic fluid remains an indication.

Experimental Approaches, Including "Noninvasive Prenatal Diagnosis"

A variety of experimental approaches to clinical and laboratory prenatal diagnosis have been, or continue to be, under trial, and for the sake of completeness we mention them here. No procedure is likely to replace amniocentesis or CVS, at least in the near future. But the counselor will certainly want to keep aware of developments in the noninvasive, or at least less invasive, prenatal diagnostic tools. Being able to separate out fetal DNA, after something as simple as the taking of a maternal blood sample, has an obvious attraction. A Dutch group have already tested consumer views, and they find considerable support, in principle, from a population of pregnant women, although somewhat less so from a comparison cohort of female masters students (Kooij et al., 2009). As for sampling via the cervical canal, women may differ in their views as to the

degree of invasiveness due to the insertion of an instrument; but the precedent of the Pap smear, and in contrast to an amniocentesis or CVS needle, might color acceptance of this approach.

Fetal Cell and Free Fetal DNA Isolation from Maternal Blood

The two important cell types that are released from the fetal tissue into the maternal circulation are the nucleated red blood cell and the trophoblast, and the latter cell releases fetal DNA into the maternal bloodstream (Dhallan et al., 2007; Maron and Bianchi, 2007). Research into the use of these materials for prenatal diagnosis is monitored by the National Institutes of Health Fetal Cell Study (NIFTY) group in the United States and the Special Advances in Fetal Evaluation (SAFE) group in Europe (Bianchi and Hanson, 2006). Various sophisticated means are employed to separate out these components from a sample of maternal blood. DNA has shown more promise than cells as the diagnostic material (Chiu et al., 2009; Lun et al., 2011). In a large study based upon 753 pregnancies at increased risk for trisomy 21, Chiu et al. (2011) applied the methodology of massively parallel genomic ("next-generation") sequencing. According to a chosen protocol applied to subset of these, they were able to identify all (86/86) affected pregnancies (confirmed on classical karyotyping), but with 2% of 146 non–trisomy 21 pregnancies misidentified as being affected.

Celocentesis.

The extra-embryonic celom, which exists during the first trimester, is a source for (nondividing) cells originating from extra-embryonic mesoderm. Given its anatomical continuity with the cytotrophoblast (Color Fig. 27–1), Makrydimas et al. (2006) comment that it could be conceived of as "a liquid extension of the placenta." The procedure has the attraction of an earlier timing (6–9 weeks) than CVS, but there is a high postprocedure miscarriage rate (Ross et al., 1997). Chatzimeletiou et al. (2005) have assessed its technical feasibility using FISH, prior to elective termination in a series of 12 pregnancies, interrogating chromosomes 3, 7, 9, 13, 16, 17, 18, 21, 22, X and Y, and the result was to hand within a few hours. Jouannic et al. (2008), in a similar series, noted the problems of low (or zero) cell numbers in the sample, and a risk of maternal cell contamination; they speculate that microarray analysis might improve the diagnostic capacity.

Cystic Hygroma and Pleural Effusion Fluid.

Cystic hygroma has a strong association with fetal aneuploidy, especially monosomy X. A concomitant oligohydramnios may make amniocentesis difficult. Fluid from cystic hygroma and pleural effusion contains lymphocytes and these cells can be cytogenetically analyzed within the timeframe of a few days. In one small series, 3 out of 4 cystic hygroma analyses showed aneuploidy (trisomy 21, monosomy X) (Costa et al., 1995).

Cervical Lavage or Cytobrush.

Trophoblast cells may migrate from the confines of the uterine cavity and enter the endocervical canal, and they can be collected for molecular analysis by endocervical irrigation and aspiration (lavage), or by insertion of a "cytobrush" (Bischoff and Simpson, 2006). The attraction, in principle, is of early (7 weeks) diagnosis and a (relatively) noninvasive procedure. Cells of fetal origin are greatly outnumbered by maternal cells, and cleanly isolating the few trophoblastic cells is a difficult challenge. One way around this problem is simply to analyze all the cells retrieved (by X, Y, and 21 FISH) and to assume that any trisomy detected would be fetal (Sifakis et al., 2010); the question of a false-negative XX result from such an approach would remain open.

Proteomic Fingerprinting.

Proteomic fingerprinting of amniotic fluid assesses the expression profile of proteins coded from specific chromosomes, or otherwise expressed in the context of a specific aneuploidy, and this could be considered as a functional assay for trisomy (Mange et al., 2008; Koster et al., 2010).

"Primum non nocere"

"First, do no harm" is a cornerstone of medical practice. Yet, almost inevitably, having a prenatal diagnostic procedure causes anxiety. Rothman (1988), in her book *The Tentative Pregnancy*, is particularly critical of what she sees as a medicalized distortion of the normal process of being pregnant. Hodge (1989) describes her personal experience of *Waiting for the Amniocentesis*, and we reproduce her letter in full:

> I drafted the following letter to the editor one week before I expected to hear the results of my amniocentesis:
>
> "I am 40 years old and 19 weeks pregnant with what will presumably be my third child. I am on the basic science faculty of a medical school. When I teach medical students about amniocentesis, I occasionally mention the difficulty for the woman of having to wait until well into the second trimester to receive her results.
>
> "I am in that situation myself now, awaiting my results. And before experiencing it, I was unprepared for two phenomena. One wasjust how difficult the wait is. Pregnancy is always a time of waiting, but now time has slowed down to an extent I did not anticipate. The other, more disturbing phenomenon is how the waiting has affected my attitude toward the pregnancy. At many levels I deny that I really am pregnant 'until after we get the results.' I ignore the flutterings and kicks I feel; I talk of 'if' rather than 'when' the baby comes; I am reluctant to admit to others that I am pregnant. I dream frequently and grimly about second-trimester abortions. In some sense I am holding back on 'bonding' with this child-to-be. This represents an unanticipated negative side effect of diagnostic amniocentesis. And all this, even though my risk of carrying a chromosomal abnormality is less than 2 percent.
>
> "I presume I am not alone in these reactions, yet I have not seen this problem mentioned in the literature, nor did my physician or genetic counselor discuss it with me. I am writing now to bring it to the attention of clinicians with pregnant patients undergoing diagnostic amniocentesis. I suggest to both clinicians and their patients that, when weighing the relative risk and benefits of prenatal diagnosis performed later (amniocentesis) as compared with earlier (chorionic villus biopsy), they not underestimate the negative effects of a 4½ month wait before the woman knows if she is 'really' pregnant."
>
> The next day, before I had mailed this letter, I received the results, and unfortunately they were the dreaded ones: trisomy 21. I have since then had the grim second-trimester abortion. From my current perspective of grief and shock, I encourage clinicians to help their patients avoid the denial described in my letter. My husband and I spared ourselves no pain by holding back emotionally. It has become a cultural expectation that one will keep one's pregnancy a secret until one has had the "all clear" from the amnio. One reasons, "If we get a bad result, we won't have to tell anyone." But I now believe that reasoning is wrong. After our bad result, my husband and I did tell everyone. Sympathy and support from our friends, family, and colleagues have helped us to survive the ordeal of aborting a wanted pregnancy. By keeping the loss a secret, we would have cut ourselves off from such support when the feared outcome did happen.

Not every couple will react this way, some preferring to keep their personal affairs private, but many will. The counselor needs to acknowledge these criticisms, and to rise to the challenge of providing a sympathetic and skilful service to clients/patients, according to their varying responses to deciding to have, to undergoing, and to waiting for the results of prenatal diagnosis, and then supporting those who do get an abnormal result. These issues are addressed in detail in *Prenatal Diagnosis: The Human Side* (Abramsky and Chapple, 2004).

A considerable fraction of pregnant women are, in any event, opposed to invasive prenatal testing. In a study of pregnant women (age 37 and older) who had not undergone prenatal diagnosis in Victoria, Australia, 33% had actively declined, with the two main reasons being concern about the safety of the test, and a conviction that they would not in any event have a termination. Another 6% had never been offered testing, these being for the most part women from minority groups, with single women also overrepresented, and it is a challenge to see that all who might wish to have the choice of prenatal diagnosis are indeed given it (Halliday et al., 2001).

A practical question is pain: the thought of insertion of a needle, or of a catheter, sufficiently deeply to sample a pregnancy, would naturally be cause for some apprehension.

Csaba et al. (2006) surveyed a number of women undergoing prenatal diagnosis in New York, asking them to quantify their anxiety ahead of the procedure (transabdominal CVS, transcervical CVS, or amniocentesis), and their perception of pain immediately afterward. In each procedure, the pain was typically seen as "mild," and three-quarters of the women thought it was the same or less painful than they had been expecting. Those who were more anxious—mostly the younger and nulliparous—felt the pain more keenly, and thus special reassurance should be given to this group.







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Chromosome Abnormalities and Genetic Counseling (4 ed.)

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Preimplantation Genetic Diagnosis

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CHROMOSOMAL PREIMPLANTATION genetic diagnosis is done in the setting of in vitro fertilization, and in principle it enables an unaffected embryo to be transferred to the uterus, some 3 to 5 days post fertilization. Thus, for couples facing a high genetic risk, the risk can be bypassed; and the prospect of pregnancy termination for the reason of genetic abnormality can be avoided.

Advances in the late twentieth century in the fields of in vitro fertilization (IVF), human embryo culture and manipulation, molecular genetics, and fluorescence in situ hybridization (FISH), set the stage for the development of preimplantation genetic diagnosis (PGD). From an essentially research-based exercise in a very few laboratories in the early 1990s, it has progressed to being, in the 2010s, a diagnostic tool available through a number of larger IVF clinics. PGD is applied in two main genetic settings: for the diagnosis of chromosome disorders, and for the detection of a mendelian condition. Initially, the two categories were distinguished by the methodology applied: FISH in the former, DNA testing in the latter; a distinction that is now blurring, as molecular methodologies advance. There has arisen a praiseworthy tradition of excellent communication between the major centers that do this work, with the majority contributing their data to an international clearing house (under the aegis of ESHRE, the European Society for Human Reproduction and Embryology), and detailed analyses of the accumulated experience of the participating clinics are documented in the annual reports of the ESHRE PGD consortium, which appear in the journal *Human Reproduction* (Harper et al., 2010b); and the International Society for Prenatal Diagnosis sponsors a regular conference devoted to PGD. Thus, new knowledge from the leading centers can translate readily into improved services to patients worldwide.

Chromosomal PGD is typically done on a blastomere (single cell) from a 6–10 cell embryo[1] at day 3. Alternative approaches are blastocyst biopsy at day 5 and polar body biopsy. The selected embryo is then transferred that same day (day 3 transfer) or maintained in culture a little longer (day 5 or 6 transfer). In principle, and barring the presence of mosaicism (a very important issue; see later), the pregnancy can proceed in the knowledge that the baby will be unaffected. There are two main categories of chromosomal PGD: focused PGD with respect to a particular parental translocation, or other rearrangement; and PGD being done as a general aneuploidy screen (PGD-AS).

Patients and Circumstances Where Chromosomal Preimplantation Genetic Diagnosis May Be Appropriate

Carriers of Balanced Rearrangements

A parent who is the carrier of a balanced rearrangement typically has a high risk to produce unbalanced embryos, as discussed at length in earlier chapters. Particularly in the context of an unfortunate reproductive history, often with several miscarriages, or with one or more terminations following conventional prenatal diagnosis of an unbalanced fetal karyotype, the attraction of PGD is obvious: only an embryo with a normal or balanced chromosomal constitution is transferred. The two main categories are reciprocal (rcp) and Robertsonian (rob) translocations.

In principle, the outcomes should be improved; and in practice, this is the case (Munné, 2005; Keymolen et al., 2009). An impressive report comes from Otani et al. (2006), who assessed PGD in 33 couples having had several miscarriages and no liveborn children, from a total of 117 pregnancies, and one of the couple being a translocation heterozygote. Thus, in their prior reproductive history (typically over several years), there had been a 100% pregnancy loss. Following PGD (an average of 1.24 cycles per patient), a total of 20/88 embryos from rob carriers, and 86/491 from rcp carriers, were diagnosed as normal/balanced, these comprising only 18% of the total (again attesting to the high genetic risk). Of the 19 pregnancies subsequently resulting from transfer of normal/balanced embryos, just one (5%) miscarried; the other 18 pregnancies had either proceeded into the second trimester or had culminated in live birth. 100% loss versus 5% is a notable contrast. This very considerable improvement does imply that many of these couples would otherwise have had no impediment to fertility (although not all couples had been able, at the time of the study, to achieve a pregnancy: as applies, of course, to all IVF).

A high rate of unbalanced gametes would logically translate into a similarly high risk for unbalanced embryos. A stringent assessment of a link between the proportion of unbalanced sperm and the outcome at PGD comes from Escudero et al. (2003). They noted that a fraction of greater than 60% unbalanced sperm was associated with a poor reproductive prognosis, and that biopsied blastomeres had a somewhat higher abnormality rate than did the sperm; and they devised a formula to estimate the relationship between these rates. The formula is $A = -55 + (1.9 \times B)$, where A is the percentage of abnormal embryos, and B the percentage of abnormal sperm. We may apply this formula to the example in Wiland et al. (2008), concerning their study of a man heterozygous for t(2;7)(p11.2;q22), he and his partner (and other family members) having suffered recurrent miscarriage. Of his sperm, 66% revealed malsegregation; whence $A = -55 + (1.9 \times 66) = 70\%$. The actual observation at PGD was 57% abnormal embryos, but this was based on the small numbers of 4 out of 7 embryos being unbalanced.[2] Had the rate been 5 of 7, the fraction would have been 71%. But in practice, sperm analysis is not routinely undertaken; the essential thing is PGD result.

Women of Older Childbearing Age and Preimplantation Genetic Diagnosis–Aneuploidy Screening

Given the maternal age effect in chromosomal abnormality, it had initially been supposed, and quite reasonably so, that PGD for aneuploidy screening (PGD-AS) would be valuable for older mothers; more often in the setting of difficulty in conceiving, or with a history of miscarriage. In other words, older mothers who might otherwise have been considering IVF would have the option of adding PGD-AS to the procedure; and eliminating embryos with a trisomy would "surely" improve their chances of a chromosomally normal baby (Munné et al., 2002a).

Apparent Poor Results from PGD-AS.

But the early promise of PGD-AS faded as more units began to offer this service. One large and stringent trial (multicenter, randomized, double-blind controlled), conducted over 2003–2007, comparing PGD with standard IVF in 408 women, of age range 35–41, showed a clear *lessening* in success (a 25% vs. 37% pregnancy rate), in those receiving PGD-AS (Mastenbroek et al., 2007). Twisk et al. (2008) reached a similar conclusion against a favorable effect due to PGD-AS. Similarly, PGD-AS from blastocyst biopsy may have *less* success than simple blastocyst transfer (Jansen et al., 2008). This paradox demanded explanation (Donoso et al., 2007).

Overinterpreted Mosaicism?

Vanneste et al. (2009a,b) accounted for these observations as follows. The rate of mitotic error during the first 3 days of postfertilization existence is so high—much higher than that due to meiotic error—that biopsy of a single blastomere may fail to give a true picture of the karyotype of the conceptus. *Most* of the cells may be abnormal: "chaotic mosaicism" (and see later). The abnormalities comprise both simple aneuploidies and deletions/duplications due to the effects of a "breakage-fusion-bridge cycle." But the small minority of normal cells may be the ones that endure, and which, following implantation, are the ones that give rise to the embryo, with the abnormal cells cast aside (Barbash-Hazan et al., 2009). According to this view, these first three waves of mitosis, that give 2, 4, and then 8 cells, may be a particularly vulnerable stage in human development; but also a stage during which, if a single biopsied cell is shown to be abnormal, the risk can be overinterpreted, and the interpretation misleading. Aneuploidy, in mosaic state, may be rather common, and many embryos might be, by the criterion of PGD-AS, rejected: and yet some could have produced a "take-home baby" (in the jargon of fertility clinics).

Some support for this interpretation might come from Hanson et al. (2009), who examined the accuracy of the chromosomal diagnosis at PGD-AS, by analyzing the constitutions of 173 embryos that had tested aneuploid at blastomere biopsy. Only 10% (the authors' comment that this correlation was "rather low" somewhat of an understatement) proved to have the exact same diagnosis that had been made at PGD. One-third did, however, have the same abnormality as at PGD, along with other abnormalities; and almost all (96%) of the embryos did have a chromosomal abnormality of some sort, albeit in many, different from the PGD diagnosis. From these data, the conclusion might be drawn that PGD-AS, as a diagnostic procedure, has been thwarted by the biology of chaotic mosaicism.

Ascertainment Bias Due to Only Discarded Embryos Being Analyzed?

Wilton et al. (2009) emphasize the very *high* diagnostic accuracy when the test endpoint is not the analysis of an untransferred embryo, but the baby. Of course, it is typically the "good-looking" embryos that are chosen for transfer, and the discarded ones whose study reveals the chaotic mosaicism. Perhaps good-looking embryos, if we could but observe their tubal journey in vivo, might, in contradistinction to the interpretation outlined earlier, quite often present a picture of nonmosaic chromosomal normality. In one review in which a number of PGD-*normal* embryos had not been transferred, none of these turned out to be abnormal (Staessen et al., 2004). And Moayeri et al. (2008) observed that the appearance of the embryo, according to the "day-3 morphology score," may, in an older mother, predict an improved chance for euploidy at PGD-AS.

Confounding Due to FISH Artifact?

Treff et al. (2010a) propose that mosaicism at PGD may be more apparent than real: simply for technical reasons inherent in the methodology, FISH may be presenting a false picture (and see section on "The Technical Challenge of FISH" that follows). If the same embryos are tested in parallel with FISH and SNP-microarray, mosaicism in the latter group falls to much lower levels, in a range of 25%–30%. Diagnosis of aneuploidy by FISH may be disturbingly inaccurate: for example, in a separate study of day 3 embryos called as trisomic by FISH, nearly half were actually euploid, when four different sections of the day 5 blastocyst (three from the trophoblast, one from the inner cell mass) were examined by microarray (Northrup et al., 2010). The normality of the trophoblast in all sections undermined the theory that aneuploid cells might be sequestered to potential placental tissue. Treff et al. (2010a) suggest that "it is becoming clear that FISH-based technology is inadequate for the diagnosis of aneuploidy in human embryos."

The Need for Clinical Trials.

Be these observations and conclusions as they may, given the disappointing results in practice from PGD-AS, an expert panel under the aegis of ESHRE (Harper et al., 2010a) has concluded that "the most effective way to resolve the debate about the usefulness of PGS is to perform well-designed and well-executed randomized clinical trials." Such trials, they recommend, should distinguish time of biopsy (cleavage stage, polar body, or blastocyst), the methodology applied (FISH versus microarray), and appropriate end-point (delivery rates). The PGD community will await the findings of such a trial with much interest. The early reports coming from SNP-array aneuploidy screening do offer grounds for optimism, and Treff et al. (2010b) claim an impressive 98.6% accuracy of diagnosis.

Recurrent Miscarriage and Preimplantation Genetic Diagnosis–Aneuploidy Screening

Some couples who are chromosomally normal, but who have had a history of multiple miscarriage, may benefit from PGD-AS (Rubio et al., 2003). The fraction of spontaneous abortion that is due to chromosomal abnormality has been revised upwards in recent years (Menasha et al., 2005, and see p. [link]); and some miscarrying couples may have been having recurring aneuploidies. Ferraretti et al. (2004) showed in a series of PGD patients presenting for repeated cycles of treatment, for the reasons of either older age (>37 years), or of past implantation failure, that previous euploidy or aneuploidy predicted future euploidy or aneuploidy. Similarly, Munné et al. (2004), demonstrated that, at least for younger (<35 years) women, a previous trisomic pregnancy predicted an increased risk for future aneuploidy. The women in this study, coming forward for PGD-AS, had only 29% of embryos euploid, on a limited range of chromosomes tested (X, Y, 13, 18, and 21, and in most cases also 15, 16, 17, and 22), compared with 52% in those having the indication of gender selection due to an X-linked condition (and whose fertility would presumably have been unimpaired). Increasing the FISH panel would enable more aneuploid embryos to be recognized as such (Lathi et al., 2008), or with microarray, the whole karyotype is examinable.

While the interpretation may be a little clouded, considering that there is a presumed meiotic basis in a previous aneuploid pregnancy, but with the possibility of a mitotic generation of a PGD aneuploidy(ies), nevertheless, the inference that PGD may have value in the setting of a history of aneuploid miscarriage is not unreasonable. The ESHRE panel mentioned earlier has taken a conservative stance and proposes that a true view of the appropriateness of PGD-AS in the setting of recurrent miscarriage requires the insight that only a randomized controlled trial would provide (Harper et al., 2010a).

Gender Selection.

Gender diagnosis at PGD, with the use of X and Y FISH probes, may be appropriate in the context of a sex-related genetic risk, whether mendelian or nonmendelian, an example of the latter being autism (Amor and Cameron, 2008).

Clinical and Laboratory Procedures

From the earlier categories of patient, two major karyotypic classes of couple are to be considered: couples one of whom carries a balanced chromosomal rearrangement; and those who have normal chromosomes, and who may have chosen "aneuploidy screening". The former group will command most of our attention, although of course they are the smaller group. Those who make the decision to embark upon chromosomal PGD—and for whom the laboratory have advised that testing, in their particular case, would be feasible—will need to enroll (if not already) in an IVF program. If FISH is to be the methodology, close liaison with the laboratory is required, in order that the scientist can have

the appropriate probes prepared and ready for use on the day. The timeframe for having hyperstimulation of ovulation is established from the woman's menstrual cycle, and ovum "pickup" is conducted by transvaginal endoscopy under ultrasound guidance. Given the high risk of abnormal malsegregants typically associated with chromosome rearrangements, it may be useful in this situation to employ a stimulation protocol designed to maximize the numbers of ova produced (Fridström et al., 2001), albeit that a concern that stimulation might of itself tend toward aneuploidy needs to be weighed (Baart et al., 2007). The ova are collected, and then exposed to sperm in vitro. If the couple are of otherwise normal fertility, simple mixing with the male partner's sperm suffices. With some forms of male infertility, and this is quite often the case in the male heterozygous for a chromosome rearrangement, ICSI[3] is needed.

On day 1, around 18 hours after exposure to sperm, the oöcytes are checked for the presence of two pronuclei and two polar bodies, as evidence that fertilization[4] has occurred. They are then returned to tissue culture medium; in a few hours syngamy will occur, and over the next 48 hours the first few mitoses will have produced cleavage-stage embryos of 6–10 cells.

Standard Day-3 Blastomere Biopsy

On day 3, in the morning, one or at most two cells (blastomeres) are removed from each embryo, under the inverted microscope.[5] This requires a hole to be made in the "shell" (the zona pellucida, which has not yet been cast off), the cells being extracted by very gentle suction. These cells are subject to FISH or microarray analysis, in order to determine whether they have a normal/balanced chromosome constitution or an unbalanced form of the rearrangement. One or two embryos shown to be chromosomally normal/balanced are then transferred to the uterus, on the afternoon of day 3 or the morning of day 4, and with luck one[6] will develop into a normal infant. The remaining embryos with a normal/balanced chromosomal complement will usually be cryopreserved, in case the first embryo transfer does not result in a pregnancy, and perhaps for a second pregnancy further in the future. The process is outlined in Figure **26–1**.

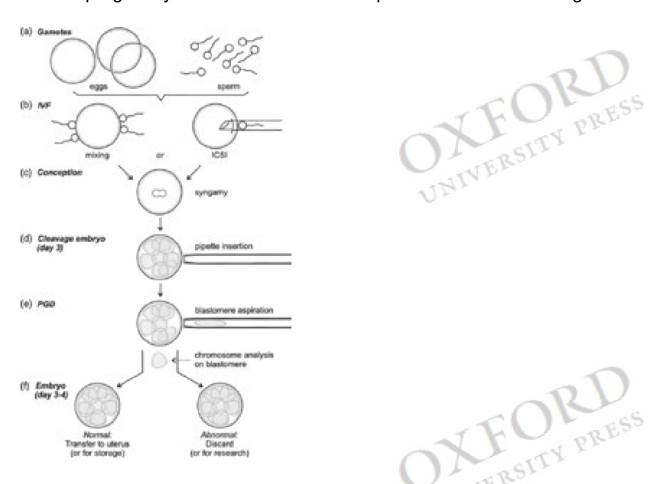


Figure 26–1
The process of in vitro fertilization (IVF) (with or without intracytoplasmic sperm injection [ICSI]) and preimplantation genetic diagnosis (PGD) at the day-3 stage. (*a*) Oöcytes are obtained from the woman, and sperm from the man (by testicular aspiration, if necessary). (*b*) Oöcytes and sperm are mixed in vitro; or, single sperm are injected into an oöcyte (ICSI). (*c*) Syngamy, the fusion of male and female pronuclei, occurs. After incubation for 3 days, (*d*) one or two[7] blastomeres are removed from the embryo, and (*e*) these cells are then subject to chromosomal analysis. (*f*) Normal (or balanced) embryos are chosen for transfer to the uterus, or possibly for cryopreservation for a future transfer.

Blastocyst Biopsy

If the embryo is incubated for 1–2 more days, it advances through the morula and early blastocyst stages. Considerable selection pressure applies during this short period, and many chromosomal abnormalities, including most monosomies and some extensive mosaics, impose a lethal burden (Clouston et al., 2002). The chances of successful transfer might, in principle, be better if the embryo has declared itself capable of developing this far, or, as Johnson et al. (2010a) put it, if it has "had the opportunity to 'self-correct.'" There might seem some attraction in delaying PGD until the blastocyst is forming, since cell number has increased, and differentiation between inner cell mass and trophoblast has begun, allowing sampling to be focused on the trophectoderm (in a tissue-origin sense, a very early chorionic villus sampling). This involves making a hole in the zona pellucida and allowing a small part of the lining of the blastocele cavity to herniate through ("assisted hatching"), and part of this tiny bulge could be excised by laser, or teased away by manipulation (Fig. **26–2**). In an array-based analysis, using embryos from a youngish cohort of couples (average maternal age = 31), Johnson et al. (2010a) examined blastocysts from which they were able to dissect out the trophectoderm and the inner cell mass. Encouragingly, almost all were concordant as to karyotype between these two tissues (and note the similar findings from Northrup et al. 2010, discussed earlier). And perhaps reflecting the younger age profile, the considerable majority (80%) of embryos were euploid. Against this approach, it is a given that the maternal genital tract offers a better environment for growth than does an IVF container, and a better chance for survival. A controversial question is whether this longer incubation in vitro might disturb the epigenetic state of the embryo.

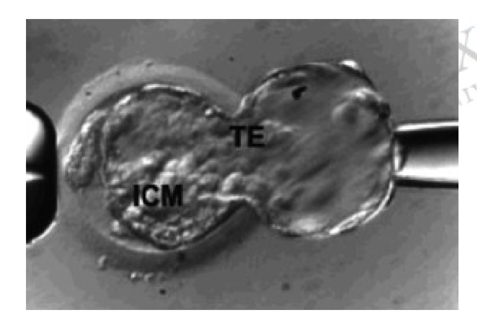


Figure 26–2
The process of blastocyst biopsy, at day 5–6. The blastocyst is held in place by the suction pipette on the left, which is applied directly to the zona pellucida (the "shell" that invests the blastocyst, "inherited" from the ovum; see Fig. **26–3**). Trophectoderm (TE) has herniated through a laser-generated hole, visible in this view at 3 o'clock in the zona pellucida; the inner cell mass (ICM) remains comfortably within the zona pellucida. Suction will be applied through the biopsy pipette (right), and about five cells from the TE gently teased off. (From S. J. McArthur et al. 2008, Blastocyst trophectoderm biopsy and preimplantation genetic diagnosis for familial monogenic disorders and chromosomal translocations, *Prenatal Diagnosis* 28: 434–442. Courtesy R. P. S. Jansen; reproduced with the permission of Wiley-Blackwell.)

Polar Body Analysis

Polar body (PB) genetic analysis (satisfyingly requiring recall of some elementary facts of biology) has been used for PGD (or "preconception diagnosis") in a few research laboratories, and legal or logistic constraints against PGD in some jurisdictions have propelled interest (Landwehr et al., 2008; Montag et al., 2009). The approach has typically been FISH based. The process of biopsy is illustrated in Figure **26–3**. PB analysis allows a focus on the vulnerable gamete, that is, the ovum, the great majority of meiotic nondisjunction occurring here. Disomic or nullisomic gametes could be identified, and thus excluded from fertilization.

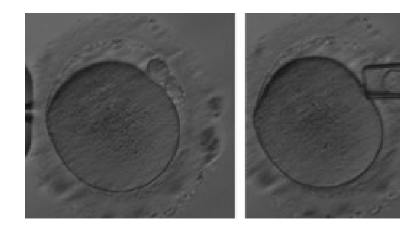


Figure 26–3
The process of polar body biopsy. (*Left*) The egg is held in place by a suction pipette, which is applied directly to the zona pellucida (the "shell" that invests the egg itself). The first and second polar bodies are located in the space between the zona pellucida and the egg's cell membrane (the latter seen in dark circular outline). The egg is manipulated so that the polar bodies are at the 1 o'clock position. (*Right*) The pipette has entered a laser-cut hole in the zona pellucida, and the two polar bodies have been aspirated into its lumen. (From M. Montag et al., 2009, Polar body biopsy: a viable alternative to preimplantation genetic diagnosis and screening, *Reproductive Biomedicine Online*, 18 Suppl 1:6–11. Courtesy M. Montag; reproduced with the permission of Elsevier.)

By way of example, imagine that the asterisked gametocyte in Figure **3–3a** (in Chapter 3) is the first PB (PB1), and that the two chromosomes shown within it are chromosome 18s: that is, PB1 is disomic 18. The "empty" gamete to the right, therefore, would be a nullisomic 18 oöcyte, and thus of course to be discarded. A nullisomic second PB (PB2) (one of the empty cells in the next row) should provide corroboration. The reader may also determine, on study of Figure 3–6 with respect to predivision of chromosomes at meiosis, why analysis of PB2 alone could in some instances mislead. The cell labeled "disomic gamete" in this figure could be the oöcyte, but PB2, represented by the cell next to it, shows a normal monosomy. Both PBs together can enable the full picture to be deduced, and the disposition of all four chromatids can be accounted for, provided probes are used that enable the distinction between single chromatids and double-chromatid chromosomes.

A euploid egg might become, after fertilization, an embryo with some aneuploid cells ("chaotic mosaicism," as elsewhere discussed in this chapter). But provided the euploid cell(s) carry on through and give rise to the inner cell mass, then this transient mosaicism will have been unknown, and unimportant. Thus, a theoretical advantage of PB diagnosis is that the uncertainty relating to aneuploidy screening at PGD, due to biopsy of a possibly unrepresentative aneuploid blastomere, could be avoided (Geraedts et al., 2010).

Landwehr et al. (2008), in a research setting, have applied PB analysis in the management of couples presenting with repeated implantation failure, or for advanced maternal age, and, using comparative genomic hybridization (CGH) and thus able to interrogate every chromosome, they discovered an aneuploidy rate of 75%. This figure is very different from the corresponding figure of 22% in the series of Fragouli et al. (2006b); differences in the reproductive backgrounds and ages of the couples coming forward likely underlie this seeming incongruity (indeed, half of the couples in Fragouli et al. were attending because of *male* factor infertility).

Segregation Analysis and FISH Probe Selection

Analysis of segregation patterns of structural rearrangements has been discussed extensively in earlier chapters. The specific PGD-related risks for reciprocal and Robertsonian translocations are noted in Chapter 5 and Chapter 7, these two forms accounting for the substantial majority of "chromosomal" PGD patients. When choosing FISH probes for a particular PGD, each and every possible segregation outcome, as noted later, must be considered. The pattern of FISH signals that each outcome would generate, and the certainty of being able to distinguish a balanced or normal chromosome constitution, need to be carefully thought through. For the common case of the autosomal translocation, and using the example of a translocation 46,XY,t(14;18), we set out in Figure **26–4** the full range of possible blastomere combinations. As noted earlier, the more embryos that can be biopsied, the better, since the odds for unbalanced embryos are high and may vary unpredictably from cycle to cycle (Fridström et al., 2001).

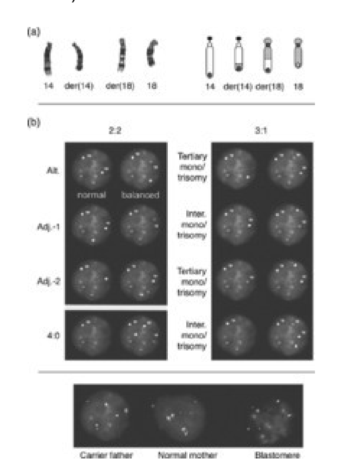






Figure 26–4
(*a*) Partial karyotype of a translocation 46,XY,t(14;18)(q24.1;q21.3) that had been the presumed basis of a series of miscarriages, leading the couple to seek preimplantation genetic diagnosis (PGD). The colored spots on the cartoon karyotype reflect those seen in the same chromosomal sites on the fluorescence in situ hybridization (FISH) analysis. (*b*) PGD using FISH for this translocation. The probes hybridize to the 14q telomere (red), the 18q telomere (yellow), and the 18 centromere (blue). *Upper*, the expected patterns of FISH probing for the 16 possible segregant outcomes (cf. Figure 5–4 in Chapter 5). *Lower*, FISH on parental lymphocytes shows the patterns of dots to be the same either in the carrier parent (father, *left*) or the noncarrier (mother, *middle*). FISH analysis of a biopsied blastomere (*right*) shows the pattern of the normal or balanced state, and thus this is a suitable embryo for transfer. The depictions of the upper panel are artificially produced; the lymphocytes and blastomere of the lower panel are actual observations. Note that in each blastomere, three dots, one of each color, are due to the gamete from the noncarrier parent, and the remaining dots represent the chromosomal

contribution from the carrier parent. (Case of E. Baker.) *See also separate color insert*.

Probes are designed which hybridize to judiciously chosen parts of the chromosomes of interest. With most simple reciprocal translocations three probes will, in general, be required: two that hybridize to a point within the translocated segments, and one to one of the centromeres. Pericentric inversions lead only to two unbalanced forms, and these can be accounted for by the use of a subtelomeric probe at either end of the chromosome, and a centromeric probe (Escudero et al., 2001). The most readily available commercial markers for recognition of the translocated segment are subtelomeric probes (Scriven et al., 1998). Observing the number and (to some extent) the disposition of colored spots in the nucleus of a blastomere removed from the IVF embryo allows a deduction of the chromosome complement. Probes to α-satellites enable rapid (2–3 hour) detection of the centromeres, although the single- or low-copy probes for telomeres take longer to hybridize, necessitating a late day 3 or early day 4 transfer (Delhanty and Conn, 2001). It is necessary to show that the probes give clear and unequivocal signals on interphase nuclei and chromosomes from the carrier parent, ahead of proceeding with IVF and PGD. If there is a living individual with the imbalanced state, the opportunity should be taken to check that the probes would give a correct interpretation. These points on probe selection are dealt with more expansively in Delhanty and Conn (2001).

The Technical Challenge of FISH.

Single (or at most two) cell FISH requires consummate skill on the part of the cytogeneticists and embryologists who do this work. Even the most technically adept scientist, however, cannot achieve 100% of resolvable FISH signal on all chromosomal targets. This is not normally a problem for other applications where many nuclei or metaphases are available for study. In PGD it is an important, and perhaps a critical limitation.

Consider the impediments to success in a “simple” PGD for a typical reciprocal translocation. Suppose three probes are to be used, each probe hybridizing to two chromosomal sites (on two normal chromosomes, or on the normal and derivative chromosomes, respectively), to give six hybridization spots in total from the normal or balanced embryo. Supposing that the hybridization efficiency of each probe is 98%, the probability of the normal or balanced embryo giving six correct signals in one cell is $(0.98)^6 = 0.89$. These facts of simple arithmetic point to the risk that a normal or balanced embryo could be diagnosed as unbalanced, and vice versa, purely because hybridization is less than 100% efficient. Further, if a second round of FISH is employed, in order to interrogate more chromosomes, the efficiency of the procedure decays, and it becomes inevitable that some misdiagnoses will be made (Wells et al., 2008). Munné et al. (2000b) estimated an average error rate of 6% based on studies of all blastomeres in a series of donated embryos, and 10% in real PGD situations; signal splitting, signal overlap, and incompletely penetrating probe may be the usual reasons. With particular reference to aneuploidy screening, Treff et al. (2010a) emphasize the potential for error due to poor spreading and fixation of cells. The most likely adverse outcomes would be wastage of normal embryos (diagnosed as monosomies) and diagnosis of embryos with a trisomy as normal. It is not surprising that centers that offer PGD for translocations usually recommend that it be followed up by conventional prenatal diagnosis to check that these sorts of error have not happened (Munné et al., 2002a). As discussed earlier, the technical capacity of FISH has been called into question, and SNP-microarray may prove to be the superior methodology.

Microarray Analysis

The first infant born following PGD-AS by array-CGH was reported in 2001 (Wilton et al.), and the first array-CGH polar body study the following year (Wells et al., 2002). The case is now being made for the microarray approach, both for aneuploidy screening and for targeted imbalances due to a parental translocation (Treff et al., 2010a, 2010b, 2011a; Alfarawati et al., 2011; Fiorentino et al., 2011). The whole karyotype is in principle examinable. Microarray does have the advantage of not requiring specific or tailor-made probes: the methodology can be by hybridization of amplified DNA to a CGH- or SNP-based array. There is the disadvantage that the time taken for analysis often means that embryos have to be cryopreserved after biopsy, pending the result, although it may be that technical advances in vitrification of embryos, and in the laboratory methodology of single-cell microarray analysis, will circumvent this question (Wells et al., 2008; Treff et al., 2009b; Vanneste et al., 2009a). An alternative molecular approach, in the specific case of a parent being a translocation carrier, is the measurement of short tandem repeat markers on the chromosomes in question, which can allow a quantitative assessment of the relevant segments, and thus distinction of euploidy versus aneuploidy (Traversa et al., 2010). In the case of polar body analysis, Landwehr et al. (2008) have developed a “rapid CGH” methodology, allowing diagnosis within a timeframe of 16 hours. Johnson et al. (2010b) have dealt with the problem of interpretation due to copy number variation by including parental DNA samples in the analytical procedure. As with FISH, the normal and the balanced carrier states cannot be distinguished.

Considerations of Embryology

The Problem of Mosaicism and “Chaotic” Embryos

The 6–10 cell embryo (the stage at which blastomere sampling is typically done) probably contains only one or two cells whose descendants will go on and form the inner cell mass, and thus, eventually, the embryo proper and the fetus. Chromosome studies on IVF embryos can reveal different chromosome constitutions in different cells, up to the point of “chaotic” embryos in which several cells each have a different aneuploidy, or collection of aneuploidies (Vanneste et al., 2009a and b), and as discussed earlier. It is not necessarily easy to guess (but intelligent guesses can be made), from the observed pattern of the different aneuploidies, what might have been the sequence of events at each individual mitosis that was able to lead to this eventual picture. Munné et al. (2002b) list these four main categories: diploid/polyploid mosaicism, chaotic mosaicism, mosaicism due to mitotic nondisjunction, and “split” mosaics with two cell lines that complement each other.

A particular vulnerability may apply to these very early mitoses, before the necessary genes for cell-cycle checkpoint control have fully swung into action, and maternal cytoplasmic factors are still being relied upon (Hardy et al., 2002; Voullaire et al., 2002). Alternatively, or perhaps additionally, there may be a male factor involved, with impairment of the embryo's centrosome function (Rodrigo et al., 2010). This could apply more particularly to cases of a severe spermatogenic defect, with a poor-quality sperm bringing a poor-quality centriole to the embryo, given that the first few mitoses make use of the centriole that came with the sperm (Silber et al., 2003).

But has the problem been overstated? As we discussed earlier, the power of the SNP-array may be bringing a clearer light, and exposing an inherent technical inadequacy in FISH methodology. Mosaicism can certainly happen, but it may be less frequent, and less chaotic, than we had thought in the early 2000s.

The Need to Consider a Full Range of Abnormal Segregant Outcomes

Postzygotic selection against aneuploid embryos has had little chance to have operated by day 3. Thus, in the case of translocation carrier parents, all possible segregant outcomes of a rearrangement may be encountered (see Fig. 5–4 and Table 5–2 in Chapter 5), and FISH probes will need to be selected accordingly. In the series of Ko et al. (2010), comprising some 1294 analyzed embryos, the fractions were as follows: alternate, 22%; adjacent-1, 23%; adjacent-2, 7%; 3:1, 25%; and 4:0, 2.5%; in the remaining 16% the segregation mode could not be determined. Somewhat similar fractions were reported in Mackie Ogilvie and Scriven (2002) and in Pujol et al. (2006); differences between the studies may likely reflect the particular nature of the translocations involved. A significant fraction with a balanced translocation complement, but a concomitant aneuploidy for one of the “common trisomic” chromosomes, namely 13, 16, 18, 21, and 22–8% of embryos in Pujol et al.—may warrant including an aneuploidy screen in the PGD analysis, and Treff et al. (2011a) have shown that this approach works. Some complete or partial autosomal monosomies, practically unknown otherwise, may be associated with “occult abortion” in the first 2 weeks post conception. Conn et al. (1999) describe PGD in the setting of a parental t(6;21), using a particular FISH strategy which covered the possibilities for chromosome 21 imbalances, and one embryo had a normal signal. This embryo was duly transferred, but with no more than a “biochemical pregnancy” resulting. In fact, this may have been interchange monosomy 6. And in the complex chromosomal rearrangement, the range of possible abnormality can be measured in hundreds; and yet, occasionally, a successful outcome is seen (Escudero et al., 2008).

Genetic Counseling

PGD is sufficiently complicated, not to mention expensive, that it will not usually be the first option for fertile couples wishing to avoid the birth of a child with a chromosomal disorder. High-risk scenarios might, however, warrant consideration sooner rather than later. Women may see access to the procedure as empowering, but equally, may find the process stressful; discarding an embryo with an unbalanced translocation, having had a child with that condition, may raise uncomfortable ambiguities (Karatas et al., 2010). For infertile couples (whether or not there is a chromosomal basis of the infertility) who require an IVF procedure to conceive, advice about a place for PGD-AS will need to be tempered by a continuing understanding of the differing relevance of meiotic and mitotic errors, and the factor of "chaotic mosaicism." Equally, counselors will need to keep abreast of the continuing debate about the potential of FISH in diagnosing, or in overdiagnosing, mosaicism, and an emerging view that SNP-array may be the superior procedure. For those particular couples presenting for PGD, on the basis that one of them carries a chromosomal rearrangement, a number of points need to be raised.

The Reasons for Choosing Preimplantation Genetic Diagnosis as an Option

Some couples may have had conventional prenatal diagnosis with successive terminations of pregnancies due to a high-risk translocation and be unwilling to face this prospect again. It may be difficult to distinguish a run of bad luck, with an optimistic outlook for the next pregnancy a realistic possibility, and therefore allowing the counselor to suggest a further natural attempt. Or the series of abnormal pregnancies may reflect a strong predisposition of that translocation to generate unbalanced gametes. Avoiding the possibility of termination following conventional prenatal diagnosis is, for those who have had that experience, a strong motivation (Lavery et al., 2002).

The Limited Success Rate

As discussed earlier, many IVF/PGD procedures do not produce the desired end result of a "take-home baby," and the figures for PGD pregnancies are fairly similar to those applying to all IVF patients. Thus, couples who would otherwise have no difficulty conceiving should weigh up the pros and cons of PGD and conventional prenatal diagnosis (Kanavakis and Traeger-Synodinos, 2002). Counselors seeing these couples need to be knowledgeable about all aspects of IVF and the PGD process, including an understanding of their local success rates.

The Specific Genetic Risk

The figures provided elsewhere in this book largely relate to the risk for an unbalanced chromosome complement in either a liveborn child or at conventional prenatal diagnosis. Naturally, the risk that an embryo at PGD will be abnormal is substantially higher. From the ESHRE data for 2005–2006, the fraction of embryos from rcp carriers that were transferable was just 19%; thus, an 81% abnormality rate. For the robertsonian carrier, the figures differed somewhat between the sexes, with 28% of diagnosed embryos transferable from the female, and 39% from the male (Goosens et al., 2008). Bint et al. (2011) reviewed findings from gamete (polar body or sperm) analysis and in the preimplantation embryo in the two major categories of rob (13/14 and 14/21), with respect to all forms of imbalance, adjacent and 3:0. High fractions were seen in the female, with similar figures for polar body and PGD: 33% unbalanced for the rob(13;14), and 40% for the rob(14;21). Lesser fractions of sperm and embryos were unbalanced from the male: 14% for the rob(13;14), and 11% of sperm and 5% of embryos with the rob(14;21). For recurrent rearrangements, risk data at the PGD stage may be pooled to give PGD-specific risk figures. Data relating to the common t(11;22)(q23;q11) are set out in Table 26–1 (and see also Table 5–2 in Chapter 5), and the rob figures just noted from Bint et al. apply to this chromosomal category.

Table 26–1. Risks of Generating Balanced and Unbalanced Embryos for Seven Carriers of the Common t(11;22)(q23;q11)

NO. OF EMBRYOS						
SEGREGATION MODE	**ALTERNATE**	**ADJ-1**	**ADJ-2**	**3:1**	**4:0**	**OTHER***
Van Assche et al. case 2 (male)	9	3	2	1	0	19
Munné et al. case E (male)	1	2	0	0	0	5
Mackie Ogilvie and Scriven (male)	9	4	1	0	1	0
Average proportions (male)	33%	16%	5%	2%	2%	42%
Van Assche et al. case 1 (female)	0	1	0	2	0	0
Iwarsson et al. case 5 (female)	5	3	0	3	0	4
Ibid. case 11 (female)	2	0	0	2	0	5
Mackie Ogilvie and Scriven (female)	0	0	0	2	1	0
Average proportions (female)	23%	13%	0	30%	3%	30%

Notes: Embryos were studied at PGD and subsequently, for untransferred embryos, at rebiopsy with as many cells as could be analyzed. These data are very scant but show the beginnings of how such information may eventually come to be accumulated. It is interesting that the favoring of alternate segregation in the male seen here is not reflected in the sperm data of the single heterozygote listed in Table 5–1 (see Chapter 5), in which adjacent-1 is the predominant mode. See also Table 5–2.

* Unbalanced but mode not analyzable; mosaicism; chaotic mosaicism; polyploidy.

Adj-1, adjacent 1; adj-2, adjacent-2.

Sources: Van Assche et al. (1999), Munné et al. (2000b), Iwarsson et al. (2000), Mackie Ogilvie and Scriven (2002).

Follow-up in the Pregnancy

Understandably, some couples will be unenthusiastic about an invasive procedure that could possibly put at risk the pregnancy in which there has been so much investment (Meschede et al., 1998b). Nevertheless, couples need to be aware that chromosomal PGD cannot provide a "guarantee," albeit that the misdiagnosis rate, for whatever reason, is very low, when good-quality embryos are transferred (Wilton et al., 2009). Prenatal diagnosis should be offered. Ultrasonography may be an acceptable, if imperfect compromise, only proceeding to CVS or amniocentesis if anomalies are detected. Maternal serum screening (p. 410) offers a further possibility, although it is necessary to take account of the fact that PAPP-A levels are less in IVF pregnancies, which might otherwise have been interpreted as an increased risk for trisomy 21 (Amor et al., 2009).

Nature May Intervene

A natural pregnancy may be achieved while the couple waits for the IVF/PGD preparations to be made. For example, the adjacent-2 karyotype shown in Figure 5–10 (see Chapter 5) came from culture of the products of conception of this couple's third miscarriage, and no normal pregnancies, the woman being a t(13;16) carrier. The outlook did not seem very promising, and plans were being put in place for IVF; but they then reported a naturally conceived pregnancy, in which amniocentesis showed a 46,XY karyotype.

The Children Resulting

Children born from a PGD pregnancy, a minority group among the large numbers worldwide of "IVFlings," appear to be of essentially normal health (Banerjee et al., 2008; Desmyttere et al., 2009). They may be born slightly earlier, and be a little lighter, than normally conceived babies. These risks, and a risk for perinatal death, may be elevated in the case of a multiple pregnancy (Liebaers et al., 2010). Perhaps unsurprisingly, for a child in whom so much has been invested, PGD infants score well on a scale of "warmth–affection" (measured by observing how infants may be cuddled and kissed, and how positively and kindly spoken to). We can anticipate longer-term studies on the health and development of these "PGD children," in the fullness of time (a present lack whereof is "deplored" by Liebaers et al.). The risk for Beckwith-Wiedemann syndrome in IVF babies generally is noted on p. 369.

Notes:

[1] We mentioned the power of language in referring to babies or fetuses, and mothers and pregnant women, on p. 418. *Embryo* is another word with a laden identity, and expressions such as "embryo destruction" can carry emotional weight. In fact, the embryo proper has yet to develop—this only happens once the primitive streak has started to differentiate. Jones and Veeck (2002) debate these fine points and propose that "pre-embryo" is a more accurate term for the period from conception through to the appearance of the primitive streak, that is, from 0 to 14 days. Another word with credible currency for the stage at about day 4 is *morula*. On about day 5, the next stage is the *blastocyst* (and see Fig. 27–1, 1 and 2), and this term is specifically applied to biopsy undertaken at this time.

[2] In this case, two of the three normal/balanced embryos were transferred, with a triplet pregnancy resulting. Two of the fetuses were monozygous heterozygous male twins; for obstetric reasons, these two fetuses were selectively terminated, and the remaining 46,XX fetus carried to term, and a normal girl born.

[3] For the record, pregnancies conceived following ICSI are not more prone to confined placental mosaicism than is the general population (Minor et al., 2006.)

[4] Since fertilization in vitro can be observed as it actually happens, the fine detail of the process can be appreciated. The first act is penetration of the ovum by the sperm. To the embryologist, this is only the prelude to conception; the true moment of conception is the point at which the male and female pronuclei fuse, their chromosomes aligning on a common metaphase plate ("syngamy"). Once that event has taken place, the zygote has come into existence. At the first mitosis, it loses that name, and becomes, in IVF parlance, a "cleavage-stage embryo," or simply an embryo (or more pedantically but perhaps usefully, as commented earlier, a pre-embryo).

[5] Removing two cells from an embryo with seven or more cells has been considered not to affect the potential of the embryo to develop (Van de Velde et al., 2000).

[6] If two embryos are transferred, this is not designed to produce twins (for whom there is an increased obstetric risk), but rather to improve the odds that one will succeed. PGD may allow a lesser number of embryos to be transferred—ideally just one ("elective single embryo transfer," eSET)—thus reducing the likelihood of multiple pregnancy.

[7] The live-birth rate is less when two cells are removed (De Vos et al., 2009).







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Chromosome Abnormalities and Genetic Counseling (4 ed.)

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chromosome abnormalities detected at prenatal diagnosis

Chapter: chromosome abnormalities detected at prenatal diagnosis
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THE MAIN FOCUS of chromosomal prenatal diagnosis has been upon trisomy 21, usually in the context of older childbearing age or of an increased-risk screening test. Trisomy 21 does remain, for most women and couples, the prime concern—the condition that most people are aware of—but with the modern ability to detect subtle imbalances on direct testing at chorionic villus sampling and at amniocentesis, the great majority of chromosomal imbalances are, in principle, diagnosable. And the increasing sophistication of screening methodologies (Chapter 24) has meant that a substantial fraction of chromosomally abnormal pregnancies are, these days, diagnosed prenatally.

Decision Making Following Prenatal Diagnosis of a Chromosomal Abnormality

To some extent, the possibility of "other abnormalities besides Down syndrome" should have been raised at counseling before prenatal diagnosis, in women presenting for amniocentesis or CVS as a routine discretionary procedure, or following an increased-risk assessment from screening, or indeed in whom a fetal abnormality is already known, on the basis of ultrasonography. But when a chromosomal abnormality is actually discovered, it is of course necessary to discuss in detail with the couple the implications of this particular abnormality and to help them decide on a suitable course of action.

Outlines of the clinical consequences of these abnormalities follow, to serve as a basis for the decisions that these women and couples need to make. In transmitting the information, the counselor is obliged to be clear and accurate about the particular abnormality, and to take care that the couples' autonomy in the decision-making process is not compromised. A decision for or against termination is the immediate one to be made. Some years ago, but their view remains valid, Engel et al. (1981) listed these factors influencing the parents' decision: their philosophy of life, their religious views, their socioeconomic status, and whether this was a first or wanted pregnancy or a later, unplanned pregnancy.

Unsurprisingly, the severity of the condition influences decision making. Drugan et al. (1990) found that 93% of parents having a prenatal diagnosis with a "poor prognosis" (autosomal trisomy, unbalanced translocation, 45,X with major anomalies on ultrasonographic examination) chose pregnancy termination, while only 27% of parents given a "questionable prognosis" (sex chromosome aneuploidy, 45,X with normal ultrasonography, de novo apparently balanced translocation or inversion) took this course. Shaffer et al. (2006a) undertook a large retrospective review (1983–2003), analyzing parental decisions in 816 prenatal diagnoses of a major aneuploidy, at a San Francisco clinic. Termination was chosen in 86% of autosomal trisomy, and in 60% with a sex chromosome aneuploidy. Of the latter, the rates of termination increased progressively from XXX (40%), XYY (57%), 45,X (65%), to XXY (70%), in parallel with a perceived severity of phenotype. The rates did not differ significantly over the 21-year period, to the slight surprise of these authors. Drugan et al. make the interesting observation that ultrasound visualization of fetal defects "in a society dominated by the television screen" can be useful in helping parents better grasp the implications of the diagnosis; although seeing an image of the actual fetus can also sharpen the ethical dilemma inherent in confronting the possibility of termination of a pregnancy (Williams et al., 2005).

Trisomy 21

Skotko et al. (2009) emphasize the need for the person conveying the news of a Down syndrome (DS) result to be well informed, whether that be a counselor, obstetrician, or other health professional (and this qualification scarcely confined to a diagnosis of DS). Ideally, the news should be given in person; but where that is not feasible, a phone call should be at a prearranged time. Parents who decide to continue a trisomy 21 pregnancy, versus those who have chosen termination, would presumably come from different points of view. Skotko et al. note that contact with a DS support group might be useful for some couples in deciding the fate of a DS pregnancy, although they do observe that few studies have assessed the views of those who have terminated a trisomic pregnancy, from whom the other face of the decision could be given a hearing. A study of health professionals in Finland showed some inconsistency in comparing the points of view of midwives and public health nurses with the options available to their patients (and the acknowledgment made that this difference could be seen as a healthy sign) (Jallinoja et al., 1999). Thus, most (79%) of these midwives and nurses agreed that all pregnant women should be offered a screening test, although only 44% personally accepted the concept of genetic abortion. An acceptance of abortion correlated with education and with a professional experience with DS patients. In the United States, Britt et al. (2000) studied 142 women who had had a prenatal diagnosis of trisomy 21, seen in Detroit over the period 1989–1998. Those who had already had children, and where the diagnosis of trisomy 21 was made earlier in the pregnancy, were more likely to choose termination.

Sex Chromosome Abnormality

The grayest area is sex chromosome aneuploidy, and views have been changing somewhat over recent decades, at least in the West, generally in the direction of a more conservative response to the news of a chromosomal abnormality (Christian et al., 2000; Linden et al., 2002; Boyd et al., 2011). In Denmark in 1986, Nielsen et al. reported that approximately 80% of prenatal diagnoses of sex chromosome aneuploidy at that time were followed by the choice of abortion. In an English/Finnish study from the same

period, termination (in about 60% overall) was more likely to be chosen in the case of the XXY and 45,X karyotypes, by younger parents with fewer previous children, and in all cases in which an ultrasonographic defect was identified (Holmes-Siedle et al., 1987). From a large survey of centers in five European countries, covering the years 1986 to 1997, the rate of choice of termination with respect to XXY was 44% (Marteau et al., 2002). In a German study over a similar period, termination was chosen by a much smaller fraction, only 13%, among parents who had been given a prenatal diagnosis of 47,XXX, 47,XXY, or 47,XYY (in contrast, just 2% of parents at the same clinic decided to continue a pregnancy with trisomy 21) (Meschede et al., 1998c). This may in part have reflected the practice of this clinic to emphasize the point that "the mean global IQ of around 90 falls well within the normal range and is compatible with a productive and socially well-adjusted life." In more recent years, a similar reduction in the choice of termination has been seen in France (Brun et al., 2004). A quite different experience comes from China, however (Liao and Li, 2008). Almost all pregnancies with a fetal diagnosis of sex chromosome abnormality are terminated. In considerable part, this may reflect the influence of the "one-child policy," with couples wanting the best outlook for their one and only child. In the specific case of 45,X and variants, from 19 registries in 10 countries across Europe from 1996 to 1998, 79% of parents chose termination if morphological abnormality, and in particular cystic hygroma, had been seen on ultrasonography, versus 42% in which the diagnosis had not been led into by an ultrasonographic defect (Baena et al., 2004).

Parental attributes may be (as in many respects!) important in influencing the eventual outcome of these children. In the experience of the Denver group, for example, the parents choosing prenatal diagnosis were often of higher socioeconomic status, and the children of those who had made conscious decisions to continue the pregnancy, following the discovery of a sex chromosome abnormality, had generally done better than those identified in population newborn surveys (Linden and Bender, 2002).

The way in which information is given has an important impact, and counselors need to be well aware of the weight that parents, in some emotional turmoil at the news they have just received, may put upon the news given them. Consider the example of 47,XXY Klinefelter syndrome. In the European survey mentioned earlier, Marteau et al. (2002) assessed responses to the prenatal diagnosis of XXY when counseling had been given by obstetricians, pediatricians, midwives, health visitors, or genetics specialists. Women counseled solely by genetics specialists were more than twice as likely (relative risk = 2.4) to continue the pregnancy versus those counseled either by other professionals or by other professionals along with a geneticist. It seems probable that these differences may reflect the style of counseling. Marteau et al. (1994) make the following distinctions in counseling types: nondirective counseling ("try to be as neutral as possible, covering both positive and negative aspects"), directive counseling for termination ("encourage termination" or "try to be as neutral as possible but overall convey more negative than positive aspects of the condition"), or directive counseling against termination ("encourage parents to carry to term" or "try to be as neutral as possible but overall convey more positive than negative aspects of the condition"). The desirability for a consistent approach, with access to accurate information, is to be emphasized, as is—of course—the requirement to enable women's choices to be well informed in the broadest sense, and for the counseling to be nondirective (Abramsky et al., 2001; Marteau and Dormandy, 2001; Linden et al., 2002). Beyond the clinic, there are support groups, public information resources, and talking with other parents, as means to become further informed about the implications of a sex chromosome abnormality (in the short period of time during which a decision must be made), and Linden et al. (2002) note the pros and cons of taking these paths; as noted earlier with respect to trisomy 21, the views of those who had previously chosen to terminate a pregnancy are less readily accessible. The prime responsibility for putting couples in the best position to make an appropriate decision lies with the counselor.

As for subsequently informing the children from the pregnancies that are continued, Sutton et al. (2006) emphasize the importance of telling them of their sex chromosomal diagnosis (specifically, Turner syndrome), and its implications, in a timely and sensitive manner, and of not "keeping secrets."

Submicroscopic "Microarray-Level" Rearrangement

Microarray analysis applied to prenatal samples is capable of detecting imbalance practically at the level of the operator's choice, according to which particular commercial or in-house chip is used (Rickman et al., 2006; Shaffer and Bui, 2007; Shaffer et al., 2008; Coppinger et al., 2009; Van den Veyver et al., 2009) (and see Chapter 2). Furthermore, the analysis can be performed on small amounts of material, and results may be obtained with a 48-hour turnaround. As noted on p. 418, microarrays can increase the prenatal diagnostic pickup, following discovery of an ultrasound defect, by 2%–4%. The other side of this two-edged sword is the fact that some microimbalances are not pathogenic and may simply reflect "copy number variation." Indeed, one commentator has written, somewhat provocatively, that prenatal array testing is likely "to produce a flood of information that is overwhelming, anxiety-producing, inconclusive and misleading" (Shuster, 2007). A response to this is to target the array: ask the right question, if we want a useful answer. That is, we can interrogate only those chromosomal segments for which precedent exists as being causative of an abnormal phenotype, assessing in particular the known microduplication/microdeletion syndromes, along with subtelomeric and pericentromeric regions (South and Lamb, 2009). Or, a little more broadly, to target, in addition, gene-dense regions, on the assumption that these might more plausibly be, when duplicated or deleted, pathogenic. Using these targeted approaches, the results of unclear clinical significance are minimal and are not substantially different than those found by routine cytogenetics (Shaffer et al., 2008; Coppinger et al., 2009a; Van den Veyver et al., 2009). Counselors who see these patients need to be quite au fait with the interpretations and to maintain close liaison with expert scientists in the field (and see also p. 274).

Mosaicism: Confined, Constitutional, and Pseudo

Mosaicism is the bane of cytogenetic prenatal diagnosis. Most times, it turns out to have been a false alarm, and the mosaicism in villus tissue or amniocytes does not reflect a true constitutional mosaicism of the embryo. This is a problem for the laboratory to resolve, inasmuch as they are able. We may list these categories: confined placental mosaicism, true constitutional mosaicism, and pseudomosaicism. A chromosomally abnormal cell line may exist only in extra-embryonic tissues (chorion, amnion), and the embryo is 46,N. This is confined placental mosaicism (CPM). CPM is encountered at CVS rather than at amniocentesis. It is uncommon that an observation of apparent CPM at CVS reflects a true constitutional mosaicism of the fetus. Stetten et al. (2004) reviewed a series of 4000 CVS studies done over the period 1998–2003, in which 29 cases (0.7% of the total) of CPM were defined. Testing of the newborns revealed two as having (low-level) true mosaicism. A long-term follow-up study (Amor et al., 2006) is noted later. In "pseudomosaicism," the embryonic and extra-embryonic tissues are all 46,N, and the abnormality arose during tissue culture in vitro ("cultural artifact").

Considerable discussion follows: but at the outset, we should emphasize that true mosaicism of the fetus is infrequently observed, and that *the majority of mosaicism identified at prenatal diagnosis, more especially at CVS, does not presage an abnormal baby*. It is important to keep this perspective in talking with parents (according to the particular attributes of the mosaicism, as we go on to discuss) and to avoid causing any more anxiety than that which, inevitably, an "abnormal" result brings.

Applied Embryology

Interpreting mosaicism obliges an understanding of the earliest events of development of the conceptus (Bianchi et al., 1993; Robinson et al., 2002). The zygote undergoes successive mitoses to produce a ball of cells (morula) (Fig. **27–1**,1). The morula then cavitates to produce an inner cyst, and it becomes the blastocyst (this is happening at the beginning of the second week post conception) (Figs. **27–1**, 2). The outermost layer of the blastocyst is comprised of trophoblast, and this tissue becomes the outer investment of the chorionic villi. The inner cell mass protrudes into the blastocystic cavity, and this will give origin to the embryo. It comprises two different cellular layers, the epiblast and the hypoblast. In a 64-cell blastocyst, most cells are trophoblasts, the inner cell mass comprises about 16 cells, within which only about 4 (epiblast) cells will give rise to the embryo itself.

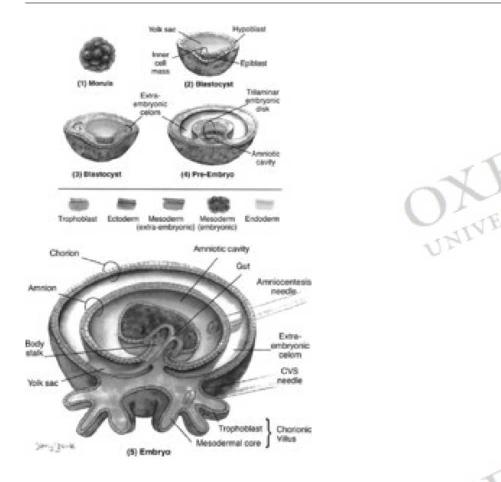




Figure 27–1
Developmental origins of tissues sampled at prenatal diagnosis (simplified). (1) Morula, 3–4 days post conception, a sphere with trophoblast cells at its surface. (2) Cross-section of blastocyst at beginning of second week, showing outer rim of trophoblast, the inner cell mass comprising epiblast (orange) and hypoblast (yellow), and the yolk sac cavity lined by an inner rim of cells of hypoblastic origin. (3) Blastocyst toward end of second week. The hypoblastic cells of the yolk sac have given rise to extra-embryonic mesoderm. Lacunae are beginning to appear in this mesoderm, and these will coalesce to form the extra-embryonic celom. (4) “Pre-embryo.” The amniotic cavity and the yolk sac bound the dorsal and ventral surfaces of the embryonic disk. The extra-embryonic celom has now cavitated the extra-embryonic mesoderm. Note that the embryonic mesoderm (middle layer of the trilaminar embryonic disk) arises from the epiblast, and thus it has a different lineage from the extra-embryonic mesoderm. (5) Composite embryo/early fetus. (Rotation has reversed the relative positions of the yolk sac and amniotic cavity.) The three embryonic tissue types (ectoderm, mesoderm, endoderm) all had origin from the epiblast, as did the amniotic epithelium. Epithelial cells from the embryo's ectodermal surface are shed into the amniotic cavity, as also are amniotic epithelial cells (both these tissues shown orange). Cells from endodermal derivatives (respiratory and urinary tracts, which originate from the gut, shown in yellow) pass into the amniotic cavity. Chorionic villi comprise mesenchymal core (of extra-embryonic mesodermal origin), gloved by trophoblast. Extra-embryonic and embryonic mesoderms are continuous at the body stalk, albeit that some embryonic mesodermal cells may then migrate into the amniotic mesoderm (Robinson et al., 2002). *See also separate color insert*.

The hypoblast forms the spherical primary yolk sac (whose roof is, transiently, the ventral surface of the embryo). The primary yolk sac gives rise to the extra-embryonic mesoderm, sandwiched between itself and the outer cytotrophoblast, thus producing a three-layered sphere. The mesodermal cells now invade the blastocystic cavity (Fig. **27–1**, 3), and this mesodermal mass is in turn cavitated to produce the extra-embryonic celom, such that there are outer and inner layers of extra-embryonic mesoderm. The outer layer, underlying the trophoblast, gives rise to the mesenchymal core of the chorionic villus, and the inner layer becomes the outer (mesodermal) surface of the amniotic membrane. The amniotic cavity enlarges at the expense of the extra-embryonic celom (Figs. **27–1**, 5, **27–2**) and eventually obliterates it (by the end of the first trimester), with the mesodermal layer of the amnion fusing with the mesodermal layer of the chorion.

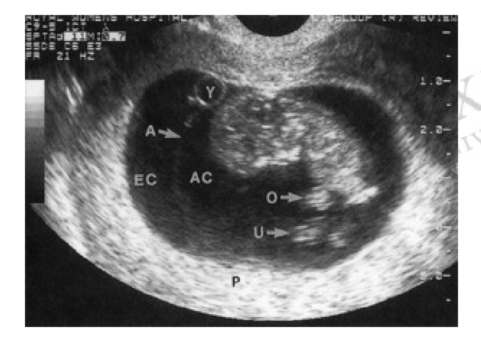




Figure 27–2
Ultrasound picture of embryo at 10–11 weeks gestational age, very close to actual size (note cm. markers at right). Note amnion (A), amniotic cavity (AC), extra-embryonic celom (EC), umbilical cord (U), “physiological omphalocele” (O), yolk sac (Y), and placenta (P). (Courtesy H. P. Robinson.) The relative positions of embryo and other structures are similar to the depiction in the drawing in Figure **27–1**, part 5.

The epiblast gives rise to the amniotic cavity, the floor of which is the “dorsal” (ectodermal) surface of the embryo, and its roof is the amnion, these being continuous at their margins. Thus, the embryonic integument and the inner surface of the amniotic membrane—which are the source of the embryonic and amniotic epithelial cells present in amniotic fluid—have the same lineage. At the beginning of the third week, the primitive streak arises from the epiblast, and this in turn gives origin to both endoderm and intraembryonic mesoderm. Endoderm gives origin, among other tissues, to urinary tract and lung epithelia, desquamated cells from which contribute to the cellular population of amniotic fluid. Albeit that the extra- and intraembryonic mesoderms have different origins, there may be migration of some intraembryonic mesodermal cells into the (extra-embryonic) amniotic mesoderm. Cells from the latter add a minor fraction to the population of amniocytes, but have a proliferative advantage, and may come to comprise most of the cells present following in vitro culture.

Amniocentesis is, therefore, a procedure that samples cells having origin from the epiblast of the inner cell mass, and these cells rather closely reflect the true constitution of the embryo. *Chorionic villus sampling*, on the other hand, samples more distantly related cells: trophoblast cells (direct and short-term culture), which were the first lineage to differentiate from totipotent cells of the morula, and villus core cells (long-term culture), which reflect the more recently separated lineage of the extra-embryonic mesoderm. The differing origins of tissues sampled by different means are set out in Figure **27–3**.

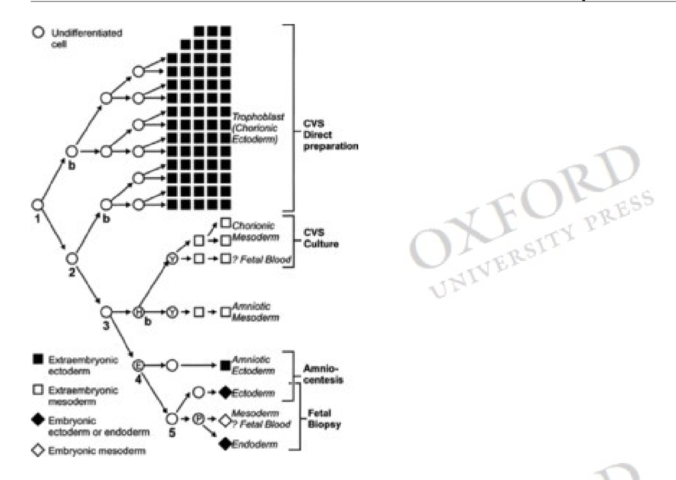




Figure 27–3
Diagram of cell lineages arising from differentiation in the very early conceptus. The fertilized egg (1) produces a trophoblast precursor (1b) and a totipotent stem cell (2), which in turn forms another trophoblast precursor (2b) and a stem cell (3) that produces the inner cell mass. The inner cell mass divides into stem cells for hypoblast (3b) and epiblast (4). The epiblast cell(s) (5) produce embryonic ectoderm and primitive streak, and the latter is the source of embryonic mesoderm and endoderm. The cell lineages sampled at various prenatal diagnostic procedures are indicated at right. E, epiblast; H, hypoblast; P, primitive streak; Y, yolk sac. (From D. W. Bianchi et al., 1993, Origin of extra-embryonic mesoderm in experimental animals: relevance to chorionic mosaicism in humans, *American Journal of Medical Genetics* 46:542–550. Courtesy D. W. Bianchi; reproduced with the permission of Wiley-Liss.) This construction is to be compared with that of Kennerknecht et al. (1993b), in which three postzygotic mitoses occur, producing eight totipotent cells, before the cells begin to take on their tissue identities. Robinson et al. (2002) propose a further variation, with some cells of the embryonic mesoderm migrating into the (otherwise extra-embryonic) mesodermal layer of the amnion.

Mechanisms of Mosaicism

Mosaicism may involve aneuploidy for an intact chromosome or for an abnormal chromosome, along with a normal cell line. Two broad formats may apply: first, a mitotic error in an initially normal conceptus which gives rise to an abnormal cell line; or, second, an initially abnormal conceptus, typically due to a meiotic error, with a subsequent mitotic event generating a normal cell line (Fig. 3–8 in Chapter 3). The distribution of the normal and the abnormal cell lines in the fetus and the placenta depends upon the time and the place of the abnormal mitotic event. If, for example, a trisomic conceptus is “rescued” by the generation of a normal cell line, at a very early stage, in a cell that is going to give rise to the inner cell mass and to some of the extrafetal tissues, then the embryo may be 46,N, and the placenta will show mosaic trisomy. If rescue occurred at a later stage, the placenta might be entirely trisomic, with a mosaic trisomy of the fetus. These and other possible combinations are depicted in Figure **27–4**. The eventual phenotype will be influenced by the tissue distribution of the cell lineages that contain the trisomic chromosome, and the normal:trisomic proportions in various tissues.

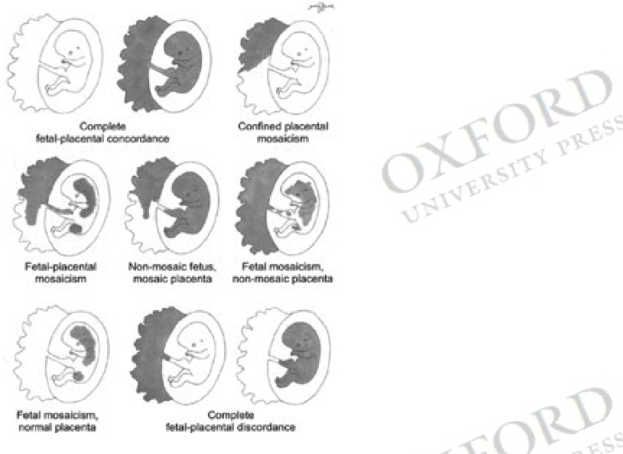


Figure 27–4
Types of mosaicism of the fetal-placental unit. Fetus depicted enclosed in its sac at right, with the chorionic villi comprising the placenta to left. Gray areas indicate an aneuploid cell line; white areas indicate karyotypic normality. In reality, the distributions of the two cell lines is unlikely to be as clear-cut as is shown here. In the examples showing placental mosaicism, the path taken by the sampling needle will determine whether the abnormality is detected or missed at chorionic villus sampling. The cartoon of the fetus, sac, and placenta is close to the form and about two-thirds of the size that actually exists at 10 weeks 0 days (gestational age as measured clinically, dated from the last menstrual period), when crown-rump length is around 30 mm.

The potential for widely differing tissue distributions of the different cell lines may confound interpretation at prenatal diagnosis. Consider the case of Jewell et al. (1992). A dup(12) chromosome was present in 87% of amnion cells, 60% of fetal blood, but only 2% of chorionic villi and in 0% of chorionic membrane. Kingston et al. (1993) provided a similar remarkable (and disconcerting) example. Amniotic fluid cells had 3% with an SMC, a sample of fetal blood showed all cells 46,N, and several tissues taken post termination had various fractions of mosaicism, including brain with 88% of cells aneuploid. Stankiewicz et al. (2001c) report an infant with the nonmosaic karyotype 46,X,der(Y)t(Y;7)(p11.32;p15.3) causing a 7p trisomy syndrome, following the CVS diagnosis of very low-grade mosaicism 46,XY[49]/46,X,der(Y)t(Y;7)[1], and yet with nonmosaic 46,X,der(Y)t(Y;7) at amniocentesis. These observations point to an early postzygotic origin of the translocation in an initially 46,XY conceptus, apparently affecting the entire inner cell mass but only a very small minority of trophoblasts. These three cases, admittedly exceptional, are instructive in emphasizing that the proportions of abnormal cells in one tissue can not necessarily be taken as indicative of proportions elsewhere.

Laboratory Assessment of Mosaicism

The resolution of mosaicism in the cytogenetics laboratory and in its clinical interpretation can differ for CVS and amniocentesis, and we will consider them separately. In terms of the laboratory result, we can apply to both CVS and amniocentesis the concept of different levels of in vitro mosaicism, originally developed for amniocentesis by Worton and

Stern (1984), and refined by Hsu et al. (1992) and Hsu and Benn (1999), as follows:

Level I.

A single abnormal cell is seen. With near certainty this is cultural artifact, and is thus pseudomosaicism, and this may be resolved after additional workup as set out in Table 27–1. The laboratory would not usually report the single cell observation, if the analysis of additional cells failed to confirm the abnormality.[1]

Table 27–1. Guidelines for Workup for the Elucidation of Possible Amniocyte Pseudomosaicism/Mosaicism

FLASK METHOD	IN SITU METHOD
A. Indications for Extensive Workup	
(1) Autosomal trisomy involving a chromosome 21, 18, 13; or 2, 5, 8, 9, 12, 14, 15, 16, 20, 22 (SC, MC) (2) Unbalanced structural rearrangement (MC) (3) Marker chromosome (MC)	(1) Autosomal trisomy involving a chromosome 21, 18, 13; or 2, 5, 8, 9, 12, 14, 15, 16, 20, 22 (SC_o, MC_o) (2) Unbalanced structural rearrangement (MC_o) (3) Marker chromosome (MC_o)
B. Indications for Moderate Workup	
(4) Extra sex chromosome (SC, MC) (5) Autosomal trisomy involving a chromosome 1, 3, 4, 6, 7, 10, 11, 17, 19 (SC, MC) (6) 45,X (MC) (7) Monosomy (other than 45,X) (MC) (8) Marker chromosome (SC) (9) Balanced structural rea (MC)	(4) Extra sex chromosome (SC_o, MC_o) (5) Autosomal trisomy involving a chromosome 1, 3, 4, 6, 7, 10, 11, 17, 19 (SC_o, MC_o) (6) 45,X (SC_o, MC_o) (7) Monosomy (other than 45,X) (SC_o, MC_o) (8) Marker chromosome(SC_o) (9) Balanced structural rea (MC_o)
C. Standard, No Additional Workup	
(10) 45,X (SC) (11) Unbalanced structural rea (SC) (12) Balanced structural rea (SC) (13) Break at centromere with loss of one arm (SC)	(10) Unbalanced structural rea (SC_o) (11) Balanced structural rea (SC_o) (12) Break at centromere with loss of one arm (SC_o) (13) All single-cell abnormalities

Notes: Criteria for extensive (A.), moderate (B.), and standard (C.) workup: A. Forty cells (20 cells from each of two flasks, excluding those cells analyzed from the culture with the initial observation of abnormality), or 24 colonies (excluding those colonies analyzed from the vessel with the initial observation). B. Twenty cells (from the flask without the initial observation), or 12 colonies (from vessels without the initial observation). C. Twenty cells (10 from each of two independent cultures), or 15 colonies (from at least two independent vessels).

MC, multiple cells (single flask); MC_o, multiple colonies (single dish); Rea, rearrangement; SC, single cell (single flask); SC_o, single colony (single dish).

Source: From Hsu and Benn (1999).

Level II.

Two or more cells with the same chromosomal abnormality in a dispersed culture from a single flask, or in a single abnormal colony from an in situ culture (i. e., possibly or probably just a single clone). Some would also include the observation of two or more colonies from the same in situ culture. The abnormality is not observed in multiple colonies from other independent cultures. This form of mosaicism is almost always pseudomosaicism. It would not usually be reported to the physician if additional workup failed to confirm the trisomy, but it may be reported if additional studies were inadequate, if fetal anomalies had been identified, or in the case of certain chromosome abnormalities which are well recognized as existing in the mosaic state (e.g., trisomy 16; see later). A course of action to resolve the issue cytogenetically, in the case of amniocentesis, is given in Table 27–1.

Level III.

Two or more cells with the same chromosome abnormality, distributed over two or more independent cultures. Level III is likely to reflect a true mosaicism, and the cytogeneticist will report this finding immediately. (Some allow level III to include more than one colony in only a single flask, although this could be an "over-interpreted level II" if two colonies in the one flask had arisen from a single cell whose progeny migrated and established separated clones.)

The distinction may not be quite as clear as this in practice, but this is a useful working definition. The mathematics of sampling comes into the picture: how many cells need to be looked at, in order to establish what level of confidence that the possibility of mosaicism of what extent can safely be disregarded? Tables have been derived to assist in answering this question (Hook, 1977; Sikkema-Raddatz et al., 1997a). Inevitably, low-level mosaicism will, on rare occasions, be missed. Given the reality that only a limited number of cells can be karyotyped, the statistics will sometimes conspire against the cytogeneticist, and only normal cells will be examined. This has to be accepted: the test is not perfect. For example, de Pater et al. (2003a) describe their experience in reporting a normal result from amniocentesis, but in due course the child proving to be a r(12) mosaic, with a high level of 50% on blood. Critically reviewing their procedures, and indeed being able to see the ring chromosome when archived material from the amniocentesis was restudied, they nevertheless drew the conclusion that their original analysis has been appropriately performed. A similar example, with respect to a CVS case, is noted later, in the section on 47,+i(5p). In CVS, the exposure to error may relate to the part of the placenta the sampling needle happens to traverse.

Newer Molecular Methodologies and Mosaicism

Mosaicism may be detected with reasonable efficiency in qualitative fluorescent polymerase chain reaction (QF-PCR). In one large retrospective study, Donaghue et al. (2005) reviewed 8983 amniocentesis and CVS samples, from which 18 cases with mosaicism were identified. More (12) were detected by QF-PCR than by karyotyping (8), although neither approach picked up all. By their reckoning, a tissue load of 15% or more abnormal cells would allow detection of mosaicism by QF-PCR. Concerning microarray, Ballif et al. (2006) tested the system, using experimental dilutions of a 46,XY sample with a 47,XY,+21 sample, in order to mimic trisomy 21 mosaicism; and they demonstrated that mosaicism of 20% or greater could confidently be identified. Similarly, Cross et al. (2007) set up mock samples from normal and trisomy 8 fibroblasts, and, by analyzing the extracted DNA with a 50K SNP array, they established that, down to a 20% level, mosaicism was readily recognized, but fading out at about 10%. In terms of

actual experience, Filges et al. (2011) detected three cases of differing forms of mosaicism among 80 high-risk pregnancies tested by array-CGH, and show, unsurprisingly, that the same dilemmas can arise with confined placental mosaicism as seen in conventional karyotyping.

Prediction of Phenotype in an Individual Case

Elegant theorizing notwithstanding, the pragmatic observations from published cases in the literature provide the mainstay of the advice that the counselor may offer the parents in an individual case. (Large series are better than single case reports, which are better than anecdote.) Are mosaicisms for some particular chromosomes, or types of aberration, of more concern than others? What is a low enough level of mosaicism, if any such exists, to have a degree of confidence that the child will be physically and mentally normal? We set out later summaries of the recorded examples from the literature, none of which necessarily provide a firm answer, but which may serve as the basis for discussion and counseling. The numbers in some are very small.

Another difficulty with these observational data is that, for the most part, the window of assessment of the child's phenotype was confined to the neonatal period. Of course, many children who are eventually diagnosed with significant handicap may have been well grown and morphologically normal at birth, with normal functional neurology (inasmuch as this may be assessed in a baby). On the other hand, it is possible to overdiagnose problems in babyhood, as a child who subsequently develops normally may prove (Warburton, 1991; Joyce et al., 2001). An important concern in mosaicism is that a cell line inaccessible to analysis—specifically, in the brain[2]—might contain the abnormal chromosome, notwithstanding a normal karyotype in the postnatal tissues which are normally examined, namely, blood and possibly skin. If so, cognitive functioning could be compromised. Those few reports that include follow-up data for some years into childhood (Baty et al., 2001; Amor et al., 2006) are therefore most valuable. Nevertheless, no certainty can be offered, recognizing that every case of mosaicism will be unique, in terms of the extent and qualitative tissue distribution of the abnormal lineage.

Chorionic Villus Culture and Mosaicism, Including Confined Placental Mosaicisim

CVS mosaicism is detected in less than 1% of procedures at the 10- to 11-week mark. (A previous figure of ~2% applied when direct/short-term culture was widely used, which favored assessment of the cytotrophoblast; but few laboratories nowadays do this analysis.) Mosaicism from an early mitotic error in a single cell can give rise to *confined* mosaicism (confined to placenta, or to fetus) or to *generalized* mosaicism (present in both fetus and placenta), according to the destined lineage of that cell; the broad range of possibilities is shown in Figure **27–4**. Depending upon the timing and site of the event producing the mosaic state, the karyotypes observed at CVS will vary. The extreme form is complete discordance, with a nonmosaic 46,N karyotype in fetus and nonmosaic aneuploidy in CVS, or vice versa.[3]

Clearly, an important distinction to make, inasmuch as it is possible to do so, is between a mosaicism confined to the placenta (CPM), and causing little or no compromise of its function, versus the presence of an aneuploid cell line extending into the fetus, plus or minus an important effect upon the ability of the placenta to support fetal development. Follow-up amniocentesis is certainly advisable: a normal result, which is very often what eventuates, will substantially provide reassurance that the aneuploidy did not involve fetal tissue. However, as a broad-brush estimate, Daniel et al. (2004) assess that on the order of 10% of CVS mosaicism for certain “rare chromosomes”[4] interpreted as CPM, may in fact reflect a cryptic fetal mosaicism, that would not be detected at follow-up amniocentesis, and which might or might not have important phenotypic consequence (see later section on “Prognosis”).

Our focus here is on confined placental mosaicism. One classification of CPM is as follows: type I, aneuploidy confined to cytotrophoblast (recognized only at direct/short-term analysis); type II, aneuploidy confined to villous stroma; and type III, an aneuploid cell line in both cytotrophoblast and stroma.

Origin of Trisomy in Confined Placental Mosaicism

Robinson et al. (1997) studied 101 cases in which CPM had been identified at CVS, seeking to establish correlates of the origin of the trisomy. Some CPM trisomies are usually of mitotic (somatic) origin, the zygote having been 46,N. Others typically arise meiotically, and the zygote was trisomic *ab initio*. That is to say, meiotic or mitotic origins of the trisomy are substantially chromosome specific. For example, trisomy 8 CPM is characteristically the consequence of a mitotic event, while in contrast, almost all cases of CPM for trisomy 16 have arisen at maternal meiosis I. From a meiotic origin, “correction” may generate a 46,N karyotype in the fetus, but there is a risk for this to be associated with uniparental disomy. Thus, of the trisomy 16 CPM cases, about half displayed UPD(16) in the fetus. A meiotic origin of the CPM typically implies a more guarded prognosis than if the error had arisen somatically. Trisomy 2 at CVS is an example of a mosaicism that conveys quite different implications according the meiotic or mitotic mechanism of its generation (see later).

False-Negative Results from Chorionic Villus Sampling

False-negative results are very rare, and more so since many laboratories no longer use direct or short-term CVS culture. False negatives are presumed to have arisen due to an early postzygotic event, such that a normal cell line is generated in the extra-embryonic tissue from a basically abnormal conceptus; or, an abnormal cell line can arise from a normal conception, and this cell line then contributing to formation of the embryo (this latter scenario documented especially in the acrocentric isochromosome; Riegel et al., 2006). The largest formal series to address this question is due to van den Berg et al. (2006). These workers reviewed nearly 2500 prenatal diagnoses from their own service and comprehensively assessed the literature. In their own material, they had no false negatives. From the literature, most false negatives have been seen in the setting of a normal short-term culture, and then either an abnormal long-term result,[5] or, if no further testing done, an abnormal pregnancy outcome. This highlights a relative instability of the cytotrophoblast karyotype, with a tendency, as the most usual scenario in this context, to lose the additional chromosome from an initially trisomic conception. From long-term CVS culture, true negatives numbered only in single figures, and several of these were likely due to maternal cell contamination. Thus, practically all of the time, a normal long-term CVS result means that the baby will be chromosomally normal.

Fluorescence in situ hybridization (FISH), applied to direct uncultured CVS, may be chosen to enable a more timely diagnosis (a faster “turnaround time,” in the laboratory jargon), and particularly in the circumstance of an ultrasound anomaly having been seen. This can target the common aneuploidies, which account for ~65% of all chromosome abnormalities. In one large series (Feldman et al., 2000), 115 direct CVS were analyzed by interphase FISH, from pregnancies in which 100 had a minor fetal anomaly by ultrasound, and 15 had a major anomaly. All of the FISH results were confirmed by routine cytogenetics, with no false positives or false negatives as compared to the results after culturing. Although the authors did not separate the chromosome abnormalities found in CVS versus amniotic fluid, overall, they found aneuploidies by FISH in 10.6% of samples, with another 3.8% of cases having chromosome abnormalities by analysis of cultured cells that had shown a normal FISH result. Thus, the common aneuploidies are highly likely to be identified by uncultured, interphase FISH, but when the result is normal, routine karyotyping (or microarray) is still necessary to detect other abnormalities.

Level III Mosaicism

Level III mosaicism in CVS raises an immediate concern. Management at this point (which will usually be around 12–13 weeks) is aimed at demonstrating, as much as possible, fetal normality; or, if it so transpires, confirming a true fetal mosaicism. Amniocentesis with rapid FISH analysis of a large number of cells, along with detailed ultrasonographic assessment of fetal morphology, is usually the next plan of action; or, in those clinics having access, a microarray analysis might be performed on the uncultured or cultured amniocytes. In fact, the majority of cases will return normal results after this additional workup, since the mosaicism is likely confined to the placenta.

A large amount of data on level III mosaicism for autosomal trisomy was gathered by the European collaborative research group on mosaicism in CVS (EUCROMIC) (Hahnemann and Vejerslev, 1997), comprising information on just over 92,000 CVS procedures from 79 laboratories during 1986–1994. Mosaicism (or nonmosaic fetoplacental discrepancy) was seen in 650 (1.5%) cases. Of these, 192 were followed up in detail, with karyotyping of fetal fibroblasts, fetal blood, amniocytes, or neonatal tissues. Most, 84% of the 192, represented CPM. The abnormal cell line was present in either trophoblast (type I CPM; in 50%), villus mesenchyme (type II CPM; in 30%), or

both (type III CPM; in 20%). A similar proportion was forthcoming from another large review, that of Phillips et al. (1996), comprising 469 cases of placental mosaicism identified at CVS in 13 separate studies, in only 50 (11%) of which was fetal mosaicism actually demonstrated. A greater risk applied when the abnormality had been detected on villus culture, and when the chromosome concerned was a marker, or one of those involved in the common trisomies. These authors emphasized the value and validity of follow-up amniocentesis.

Different Trisomies

Certain CVS trisomies are more or less likely to reflect the same trisomy in the fetus, and the pattern and distribution of the cell lines are also indicative, as set out in Table 27–3. Trisomy 21 mosaicism on CVS is the most likely to represent a true fetal trisomy 21, whether in the nonmosaic or mosaic state. A risk applies also with trisomies 8, 9, 12, 13, 15, 18, and 20. On the other hand, CPM or fetoplacental discrepancy for trisomies 2, 3, 5, 7, 10, 11, 14, 16, 17, and 22 was never, in the EUCROMIC series, confirmed at fetal or postnatal studies. In some trisomies, a true fetal mosaicism may exist, but at such a low level that there might be no discernible effect upon the phenotype. Klein et al. (1994) reported such a case, a child born of a pregnancy in which trisomy 8 was observed in 81% of CVS cultured cells, 0% of amniotic fluid cells, and in 60% of a placental biopsy at delivery: the child had 4% and 1% mosaicism in blood at 2 and 7 months of age and 0% on a skin fibroblast study, and was normal in appearance, growth, and developmental progress at age 30 months. Of course, any fetal morphologic defect shown on ultrasonography would indicate the very substantial probability of a major degree of true fetal mosaicism, and in that case the choice of termination is appropriately offered.

Table 27–3. Outcomes in 11 Cases of Detection at Amniocentesis of a Supernumerary Marker Chromosome That Turned out to Be of X or Y Chromosomal Origin

KARYOTYPE	OUTCOME
45,X/46,X,r(X)	t.o.p.
46,X,mar(X)	Normal liveborn
45,X/46,X,der(X)	t.o.p. (normal male)
45,X/46,X,mar(Y)	Normal male liveborn
45,X/46,X,der(Y)	Normal male at 1 year
45,X/46,X,idic(Y)	t.o.p.
45,X/46,X,psudic(Y)	Normal male liveborn
45,X/46,X,i(Yp)	Male, hypospadias, testicular defect*
45,X/46,X,i(Yp)	Normal male
45,X/46,X,i(Yp)	t.o.p. (normal male)
45,X/46,X,r(Y)	Normal male liveborn

Note: Infertility would be probable in at least some of the apparently normal males.

* Absence of germinal cells of seminiferous tubules.

t.o.p., termination of pregnancy

Source: Schwartz et al. (1997).

Randomness of Sampling

The vagaries of sampling may influence the interpretation, as the following examples show. We followed to term a woman in whom first-trimester CVS had shown trisomy 7 mosaicism with 47,XY,+7 in three out of eight clones; and yet three out of four placental samples (one from each quadrant), and peripheral blood from the (normal) baby, karyotyped 46,XY. Just one placental sample, which was not histologically distinguishable from the others, was 47,+7 (Watt et al., 1991). Presumably, the CVS sampling catheter had traversed this unrepresentative region of the placenta, and most of the sample that was eventually analyzed came from here. Similarly, in a case of i(5p) diagnosis at CVS (see later section on "Autosomal Isochromosome"), following the birth of the (normal) baby, we identified a region of placental mosaicism (Clement Wilson et al., 2002). De Pater et al. (1997) did a CVS in a pregnancy of 37 weeks gestation in which severe growth retardation and a heart defect had been identified, and this showed nonmosaic trisomy 22. However, from a simultaneous amniocentesis, only two out of ten clones were 47,XX,+22, the other eight being normal; and a cord blood from the (abnormal) baby gave a nonmosaic 46,XX karyotype. Skin fibroblasts demonstrated mosaicism, 47,XX,+22[7]/46,XX[25]. Of 14 placental biopsies studied by interphase FISH, only one showed trisomy 22 cells, and at a low (about 20%) percentage. Again, it may be that a small focus of trisomic tissue happened to be in the path of the CVS sampling needle, and the sample was aspirated while the needle was at this very spot. (This case is an example of "fetal-placental mosaicism," as illustrated in Fig. **27–4**.)

Prognosis

The child subsequently born provides the direct evidence of a harm, or not, due to CPM. Amor et al. (2006) undertook a detailed postnatal follow-up, from ages 4 to 11 years, of 36 children from a "CPM pregnancy," and compared their outcomes according to a number of criteria, with a control group of 195 children having had a normal chromosome result from prenatal diagnosis. The mosaicisms included trisomies 2, 7, 8, 12, 13, 17, 18, 20, X aneuploidies, markers, and one translocation. The children from the CPM pregnancies did just as well in terms of general health, development, behavior, and intrauterine growth as did the control group. Only in respect of postnatal growth was there a small difference in favor of the control group, their mean percentiles for height and weight being 64.0 and 66.4, and with the CPM children, 51.6 and 56.8 (this might have been an effect of subtly compromised placental function). These authors did note a statistically significant increase—which does not necessarily equate to biological significance—in CPM children being perceived by their mothers as "more active," and they were suitably cautious about this observation.

There have been a very few examples of presumed CPM suspected at prenatal diagnosis, a normal follow-up amniocentesis, but with the birth subsequently of a child with the same mosaicism (Stetten et al., 2004). In these, the mosaicism was, in retrospect, clearly *not* confined to the placenta, but was in fact a true fetal-placental mosaicism (Fig. **27–4**).

Uniparental Disomy

A specific concern, when CPM for a trisomy is diagnosed, relates to uniparental disomy (UPD) (Kotzot, 2008b). This is an issue in those trisomies involving an imprintable chromosome (namely, 6, 7, 11, 14, 15, 16, and 20). The embryo may "correct" by postzygotic loss of the additional chromosome, while the placenta remains partly or wholly trisomic. In the particular case of trisomy 15 on CVS followed by 46,N at amniocentesis, the Prader-Willi/Angelman methylation test can be applied. UPDs of the other chromosomes seem mostly to be without phenotypic effect per se, excepting the unlikely possibility of isozygosity for a recessive gene. Where the UPD concerned is associated, or possibly associated, with a major clinical phenotype (and see Chapter 22), prenatal testing for these UPDs (paternal upd6, upd7mat, upd11p, upd14mat and pat, upd15mat and pat, upd16mat, and upd20mat) is justified. Irrespective of imprinting, there remains also the question of a small residual trisomic cell line in the fetus, potentially contributing to an abnormal phenotype.

Effect upon Placental Function

If a cytogenetically abnormal cell line is confined to the placenta, does this have any implication for placental function? A global statement cannot be made: some trisomies may matter, and others not, and the fraction of placenta carrying trisomic tissue is an important variable. But for several trisomies at least, a placenta that is in part trisomic apparently retains a sufficient, or nearly sufficient level of function, and mostly the (46,N) fetus is satisfactorily supported. Lestou et al. (2000) analyzed a series of 100 placentas from pregnancies producing a "viable and nonmalformed" infant, using the methodology of CGH with confirmatory FISH, and found one with CPM in only trophoblast (trisomy 13), two with CPM in the stroma (trisomies 2, 12), and two with mosaicism in both compartments (trisomies 4, 18); thus, in these cases, the CPMs were apparently harmless. However, Robinson et al. (2010) observed placental autosomal trisomy (trisomies 2, 7, and 13) in 10% of pregnancies complicated by intrauterine growth retardation (and in some of these there was also the maternal complication of preeclampsia), but in none of 84 placentae from uncomplicated pregnancies. The more commonly observed CPMs at prenatal diagnosis involve chromosomes 2, 3, 7, and 8 (which mostly arise mitotically), and chromosomes 16 and 22 (mostly of meiotic origin, and typically affecting both trophoblast and villus core placental constituent parts). It is mostly CPM of meiotic origin that is associated with a risk for pregnancy complication (Kalousek, 2000). A quite different question is mosaicism with "placental mesenchymal dysplasia," in which there is a normal and a uniparental cell line (p. 396).

Amniotic Fluid Cell Culture and Mosaicism

A mitotic error in epiblast may produce mosaicism of both embryonic and amniotic tissue. A mitotic error in extra-embryonic epithelium causes mosaicism confined to the amniotic membrane. An in vitro cell division defect causes pseudomosaicism. Separating confined placental mosaicism and pseudomosaicism from true mosaicism is critical, but by no means straightforward. The distinction is, in the first instance, based upon the number of abnormal cells seen, and whether one or more than one presumptive abnormal clone exists, according to the three levels I-III set out earlier. Level I mosaicism is seen in 2.5%–7% of amniocenteses, level II in 0.7%–1.1%, and level III in about 2 per 1000 amniotic fluids (Wilson et al., 1989).

Once the laboratory studies are completed, the cytogeneticist will provide an opinion about the level of mosaicism, taking into account technical aspects of the cultures. There is generally no point, and indeed it could be counterproductive, to report level I mosaicism. The only exception would be a single cell of a clinically relevant trisomy, and if the laboratory could not perform sufficient analysis, because of limited sample, to exclude substantial mosaicism. Some level II mosaicism and all level III mosaicism do, however, require to be conveyed to the patient, carefully and clearly interpreted.

Level II Mosaicism

Level II mosaicism reflects a true fetal chromosomal abnormality in only 1% or less of cases (Worton and Stern, 1984; Ledbetter et al., 1992; Fryburg et al., 1993; Liou et al., 1993). The nature of the "mosaic chromosome" is important. If it is one that has been recorded, in life, in the nonmosaic trisomic state, or in the mosaic state, the level of concern is higher. This includes, for example, mosaic trisomies 8, 9, 13, 14, 15, 18, and 21 and mosaic isochromosomes 5p, 9p, 12p, and 18p. Albeit true mosaicism for many of the other trisomies has been observed in the malformed fetus in a pregnancy advancing well into the third trimester or in an abnormal liveborn child, these cases are so rare that a level II amniotic fluid mosaicism is still more likely due to artifact than a true significant fetal mosaicism. High-resolution ultrasonography provides helpful information in this context.

If further cytogenetic investigation is judged desirable—and it often is—repeat amniocentesis for interphase FISH analysis is the procedure of choice, with probe choice according to the chromosome in question. A large number of cells can be analyzed, and quickly. Fetal blood sampling, formerly the mainstay, is rarely used nowadays. (It is to be noted that not all mosaicism is necessarily present in blood, and for example fetal blood sampling only infrequently, if ever, detects a mosaic cell line in trisomy 5, 12, or 20, or i(12p); Berghella et al., 1998; Chiesa et al., 1998.)

Strictly speaking, no amount of investigation could ever completely exclude the possibility of a true mosaicism of the fetus, albeit the distribution of the abnormal cell line may be rather limited and possibly of unimportant phenotypic consequence. We have seen, for example, a case of level III 47,XX, +13/46,XX mosaicism at CVS, followed by the demonstration of very low-level mosaicism at amniocentesis (1/28 colonies trisomic) and fetal blood sampling (1/400 cells trisomic). At birth, a cord blood sample from the baby showed 47,XX,+13 in 1 out of 150 cells; 2/32 cells were trisomic in amnion and 1/30 and 3/30 in two placental villus biopsies (Delatycki et al., 1998). It only needed the colony from one amniocyte not to have been analyzable, or one lymphocyte to have been passed over at each blood sampling, for the true state in the baby to have gone unrecognized. The child was reviewed at age 13: she is an above average student and unremarkable on clinical examination; on analysis of 400 cells (blood and buccal cell), none showed trisomy 13 (M. B. Delatycki, personal communication, 2009). Rare similar examples exist to disquiet the counselor (Terzoli et al., 1990; Vockley et al., 1991), but a sense of perspective is to be kept: for each autosome, only the tiniest number of level II mosaicisms (zero for most chromosomes) have turned out to reflect, in fact, a recognized true mosaicism of the fetus.

Level III Mosaicism

Hsu and Benn reevaluated the issues in 1999, and they have set forth useful guidelines. These are presented in detail in Table 27–1. While every autosome has now had a mention as a mosaic trisomy at prenatal or postnatal diagnosis, some are very rare, and others are of questionable significance. Some reported associations may not necessarily have been causal. Hsu and Benn propose the stringent requirement that, before embarking upon an extensive workup, there be in the literature, for the particular chromosome, "two, or more, well-documented independent reports of confirmed amniocyte mosaicism with abnormal pregnancy outcomes." The most extensive data treating the question are published in two reports from a collaboration of a number of American and Canadian laboratories: Hsu et al. (1997) with respect to the rare trisomies and Wallerstein et al. (2000) on trisomies 13, 18, 20, and 21. We make much use of this material in the commentaries later, and every prenatal diagnosis laboratory will want to have a copy of these papers readily at hand. Ultrasonography provides useful adjunctive evidence, but apparent normality cannot be taken as a guarantee. Studies for uniparental disomy may need to be considered in the case of mosaicism for chromosomes known to be subject to imprinting. Further modifications to these guidelines can be anticipated, as new data come to hand.

One should always attempt to confirm a diagnosis of mosaicism, either on multiple fetal samples following pregnancy termination, or on blood and placenta in an infant. A posttermination study that did not confirm the abnormality could cause parents great distress, and it needs deciding with them beforehand whether they wish to have the results. An unconfirmed abnormality could be misleading in a twin pregnancy in which the diagnostic sample had come from a vanishing abnormal twin, but the posttermination tissue had come from the normal co-twin (Griffiths et al., 1996). Fejgin et al. (1997) refer to the "hopeful possibility" of mosaicism as a comfort to parents, with the posttermination tissue having sampled only the normal cell line. It is true that even multiple tissue sampling cannot be taken as having ruled out mosaicism, and a diagnosis of "apparent phenotypic normality" in a fetus still leaves open that a functional brain defect could have come to pass.

Twin Pregnancy

Discordant Karyotypes.

Discordant karyotypes may be observed in the setting of either dizygous (DZ) or monozygous (MZ) twinning (p. 33). Selective termination of the abnormal twin is an option, albeit one that cannot assure that the normal twin will be unharmed; because twins may share circulations, the process of termination of the affected twin may lead to exsanguination of the normal one (Lewi et al., 2006). In MZ twins in which "trisomic rescue" has been the basis of one twin being karyotypically normal, a risk for UPD applies, and this would be a concern in the case of a "UPD-vulnerable" chromosome.

Specific Abnormalities

In this section we attempt to outline the risks for phenotypic abnormality of specific chromosomal abnormalities detected at prenatal diagnosis. Since the available data often derive from terminated pregnancies in which only major anomalies are recognized, many of these risk figures may be underestimates. For example, a trisomy 21 fetus may appear normal, to the inexpert eye, on external observation, but we naturally assume mental defect would have resulted; and the same may apply to several other chromosomal imbalances. New knowledge will continue to accumulate, and what appears here is printed on paper, not in stone.

The small number of aneuploidies that may exist in the true nonmosaic state are noted first. In the mosaic list, almost every chromosome is represented, although in the CVS section we do include also a few instances of nonmosaicism.

Autosomal Trisomy, Nonmosaic

Trisomies 13 and 18 (and extremely rarely 8, 9, 14, and 22) are practically the only nonmosaic autosomal trisomies besides +21 that are detected at amniocentesis. Others occur but virtually all miscarry before the usual time of amniocentesis. Chorion villus sampling (CVS), on the other hand, is done at a gestational stage when a number of trisomies destined to abort have not yet done so.

Trisomies 13 and 18

There is a high likelihood of spontaneous abortion after amniocentesis, and presumably it is somewhat higher if detection is by CVS. Earlier figures due to Hook (1983) are 43% for trisomy 13, and 68% for +trisomy 18; more recent data from Won et al. (2005) indicate a rate of fetal death in utero following amniocentesis-proven trisomy 18 of 32%, while Yamanaka et al. (2006) arrived at a figure of 27%. Data for survival of a liveborn child are due to Vendola et al. (2010), as set out in Table 27–2. But the outlook for a liveborn child is so bleak, with inevitable profound mental deficiency, barely a vestige of social response in those few who survive beyond early infancy, and typically a requirement for full nursing care, that termination is sought by the majority of couples. Those who decide to maintain the pregnancy should know of the high perinatal and early infant mortality, the high likelihood of congenital malformation, and the rarity (but not impossibility) of survival beyond infancy (Brewer et al., 2002; Iliopoulos et al., 2006). Many would regard life-sustaining emergency surgery to the newborn as inappropriate (Bos et al., 1992); Carey (2001) emphasizes the need to bring the parents fully into the making of any decisions.

Table 27–2. Probabilities of Survival to 1 Week, 1 Month, and 1 Year, for Liveborn Infants with Trisomies 13, 18, and 21

	1 WEEK	1 MONTH	1 YEAR
Trisomy 13	0.42 (0.06–2.93)	0.20 (0.03–1.41)	0.03 (0.00–0.22)
Trisomy 18	0.52 (0.07–3.69)	0.30 (0.04–2.10)	0.03 (0.00–0.19)
Trisomy 21		0.98 (0.97–0.99)	0.95 (0.93–0.96)

Note: 95% confidence limits in brackets.

Source: Vendola et al. (2010).

An exceptional case exists in Chen et al. (2004e), in which nonmosaic translocation (or isochromosome) trisomy 13 was identified at CVS, but with only minor ultrasonographic findings, and mosaicism shown in subsequent amniocentesis (77%) and fetal blood sampling (14%). The child in due course had a major (but correctable) heart defect, some minor anomalies, and growth and development were judged normal at 8 months of age. Postnatal karyotyping of the placenta (46,XX,der(13;13)/46,XX/45,XX,-13), and postsurgical karyotyping of tissues from the child (46,XX), suggested that the initial chromosome constitution had been trisomy 13, but then with correction in a lineage which substantially contributed to the embryo.

Trisomy 21

We expect most readers will have an expert appreciation of the predicted Down syndrome (DS) phenotype, but we do recommend Hunter's (2001) review as a full and balanced account. Marteau et al. (1994) appraised the views of obstetricians, geneticists, and genetic nurses to the prenatal diagnosis of DS and recorded some striking differences. The respective proportions who would counsel nondirectively (see definitions earlier) were 32%, 57%, and 94%, and the respective proportions counseling directively in favor of termination were 62%, 40%, and 7%. About 6% of obstetricians would counsel directively in favor of continuing the pregnancy, but practically no geneticists or genetic nurses would.

Having received a positive 47,+21 result, what personal factors influence the parental decision? A 7½ year study, over 1989–1997, reports the views of 145 women in Michigan (Kramer et al., 1998). Most (87%) elected to terminate the pregnancy. The decision did not differ according to parity, race, religion, nor, perhaps surprisingly, with the presence or absence of ultrasonographic abnormality. Older mothers, those who had already had children, and those whose prenatal procedure was done at an earlier gestation, were more likely to choose termination. A point to be aware of is that, with modern management, the survival of DS individuals approaches that of the general population (95% surviving at 1 year, according to recent data from Texas; Vendola et al., 2010, and see Table 27–2), but comorbidities become prevalent with age, raising questions of practicalities of care as the parents themselves age (Glasson et al., 2002). On the other hand, if fetal ultrasonography shows a heart malformation and/or growth retardation, fetal death in utero or postnatal death is probable (Wessels et al., 2003). In the study of Won et al. (2005), the rate of fetal death in utero after an amniocentesis diagnosis of trisomy 21, in 392 women who decided to continue the pregnancy, was 10%.

Other Autosomal Trisomy, and in Particular Trisomies 9, 10, 20, and 22

Never (almost) do other nonmosaic true fetal trisomies survive through to a stage of extrauterine viability. Schinzel (2001) catalogs no more than about two dozen each of trisomy 9 and trisomy 22 and barely one or two of possible trisomies 7, 8, and 14, with survival through to the third trimester. Miscarriage is nigh on inevitable, usually within the 8 to 14 week gestation range. An example is *trisomy 10*, of which very rare examples as nonmosaics at prenatal diagnosis are known, but survival to term is seen only in mosaic

forms, and these infants very abnormal (Hahnemann et al., 2005). If natural abortion has not already occurred by the time the chromosomal result is received, and if there is supportive evidence otherwise, such as ultrasonographic defect, for there being a true fetal involvement, termination is appropriately offered. Schwendemann et al. (2009) reviewed the sonographic findings of fetuses with nonmosaic *trisomy 9*; heart defects and central nervous system malformations were the most frequent anomalies seen. Concerning nonmosaic *trisomy 20*, Stein et al. (2008) record five cases at prenatal diagnosis, the indication in each being the discovery of an anatomical abnormality on ultrasound, with early deaths in all except their own case, a child who in fact turned out to be mosaic on analysis of postnatal tissues. Of all the other nonmosaic trisomies, it is only with *trisomy 22* that there might be, very rarely, the possibility of a term pregnancy, and in some, limited postnatal survival (Tinkle et al., 2003; Mokate et al., 2006; Barseghyan et al., 2009).

Autosomal Trisomy, Mosaic[6]

Detection at Chorionic Villus Culture (in Some Cases Followed by a Normal Karyotype at Amniocentesis)

The substantial majority of mosaic trisomies for a single autosome are followed by a normal result at amniocentesis and at karyotyping of the child (or of the aborted fetus). In the EUCROMIC study, there were 192 gestations with mosaic or nonmosaic feto-placental discrepancy for an autosomal trisomy, and in 84% CPM was confirmed. For mosaic trisomy 8, 9, 12, 15, and 20, only a single case of each was subsequently identified with aneuploidy in the fetus/child, compared with two each for chromosomes 13 and 18, and as many as seven for trisomy 21 (Hahnemann and Vejerslev 1997). With respect to mosaicism for multiple (>1) autosomal trisomies, the presence or absence of a normal cell line is the key point: a fetal involvement is practically never seen if there is a normal cell line, and practically always seen if there is no normal cell line (M. D. Pertile, personal communication, 2002).

The general rule that Robinson et al. (1997) advance is this: CVS mosaicism due to a preconceptual (meiotic) error conveys a significant risk for fetal trisomy/UPD, whereas a postconceptual (somatic) error is usually innocuous. Mosaic trisomies 15, 16, and 22 are mostly in the former category, for example, while trisomies 3 and 7 are typically of mitotic origin, and mosaic trisomy 2 can be either.

The possibility remains for a residual effect due to *(1)* undetected (and presumably low-level) mosaic trisomy of the fetus; *(2)* uniparental disomy of the fetus; and *(3)* placental dysfunction as a consequence of a regional placental trisomy. The risks for these scenarios differ for different chromosomes, and we provide specific commentaries following. A rich source of information is the United Kingdom Association of Clinical Cytogeneticists database, at http://acccvs.ncl.ac.uk, which assembles the results and findings from practically every U.K. CVS laboratory over the period 1987–2000 (Wolstenholme et al., 2006).

Uniparental disomy may be a concern, when an "imprintable" chromosome is involved. Kotzot (2008b) proposes that upd14pat, and upd15pat or mat, warrant testing, when a risk exists. The case is less certain with respect to paternal UPD for 11p, upd14mat and upd16mat, given the variability of the phenotypes. Although the milder clinical pictures associated with upd6pat and upd7mat might be seen as making a lesser case for investigation, clinical management can be anticipated with upd6pat, and parents may want to understand the possible clinical outcome for upd7mat. Otherwise, uniparental isodisomy for any chromosome might rarely lead to an autosomal recessive disorder, but this is scarcely predictable in an individual case (unless there should happen to be a family history of a specific recessive condition on the chromosome of concern).

Mosaic Trisomy 2 at Chorionic Villus Sampling.

Two broad groups of trisomy 2 mosaicism are recognized (Robinson et al., 1997; Albrecht et al., 2001; Wolstenholme et al., 2001b). In the first, a majority (~90% of the total) are characterized by a small fraction of trisomic cells, and usually seen only in cultured mesenchymal cells. The pregnancy outcome is typically normal; in the series of Sago et al. (1997), 11/11 pregnancies had a normal outcome. It may be that these cases reflect a postzygotic generation of the trisomic lineage in a restricted region of chorionic tissue in an otherwise normal conceptus, and this small trisomic region has no discernible effect upon placental function. The second, minority group is presumed due to trisomy "correction" in a 47,+2 conceptus, from either a maternal or paternal error. The level of trisomic cells in the CVS is typically high, up to 100%, with the involvement of both trophoblast and the mesenchymal core. The placenta being substantially trisomic apparently compromises its function, and IUGR is a frequent observation, with a poor outcome (Roberts et al., 2003). One case is known of the very severe defect of "body stalk syndrome" associated with mosaic trisomy 2 at CVS (Smrcek et al., 2003).

Mosaic Trisomy 3 at Chorionic Villus Sampling.

In the EUCROMIC study, of 10 cases with trisomy 3 at either short- or long-term culture, none proved to have fetal involvement, apart from one child with a normal karyotype at amniocentesis and a very low 1/100 trisomy 3 count on blood as a newborn (Hahnemann and Vejerslev, 1997). Zaslav et al. (2004) identified a case of trisomy 3, in which the initial amniocentesis showed 47,XX,+3[8]/46,XX[27], and a repeat procedure 47,XX,+3[1]/46,XX[18]. Fetal blood was normal in 100 cells. The baby was apparently normal at birth, except for IUGR. FISH of placenta demonstrated the trisomy 3; thus, it would likely have been found by CVS, had this procedure been performed.

Mosaic Trisomy 4 at Chorionic Villus Sampling.

This is very rare; there were none in the EUCROMIC study. Two cases are recorded in Kuchinka et al. (2001). In one case, subsequent amniocentesis gave a 46,XX karyotype, but fetal demise occurred at 30 weeks, associated with considerable growth retardation (although no externally observable malformations). Upd(4)mat was demonstrated. It remains open whether the unfortunate outcome was the consequence of the UPD or due to placental trisomy. The second case did not proceed to amniocentesis; biparental disomy 4 was demonstrated in the child. Follow up at 1 year raised some reservation: although development was judged to be normal, growth indices were low, including a head circumference at about the 3rd centile (in other words, borderline microcephaly). To complicate the story, mother and child carried a balanced t(10;15). The case in Marion et al. (1990), and followed up several years later (Brady et al., 2005), was actually an amniocentesis diagnosis, but since postnatal studies showed trisomy 4 mosaicism in the placenta, it is not unreasonable to consider this is a potential CVS example. The child, at age 14, had a low-normal intellect, and some physical body asymmetries (of hand, ear, and breast). Blood was 46,XX; skin biopsy confirmed constitutional +4 mosaicism. In another case, Gentile et al. (2005) identified mosaic trisomy 4 by amniocentesis (22% of cells). The pregnancy presented at 22 weeks gestation with micrognathia, abnormal brain development, and spinal and cardiac defects. At termination, trisomy 4 mosaicism was confirmed in placental and fetal skin cultured cells; the cord blood karyotype was normal. Molecular analysis excluded uniparental disomy of chromosome 4, but showed that the trisomy 4 was of maternal meiotic origin.

An extraordinary example of mosaic trisomy 4 at CVS with double mosaicism for trisomies 4 and 6 at amniocentesis, 47,XY+4/47,XY,+6/46,XY, is described in Wieczorek et al. (2003). The double trisomy mosaicism was confirmed on skin (but not blood) karyotyping in the child, whose phenotype, while certainly abnormal, was less so than might have been anticipated.

Mosaic Trisomy 5 at Chorionic Villus Sampling.

Only three cases are recorded in the EUCROMIC study; in none was a fetal trisomy subsequently shown (Hahnemann and Vejerslev 1997).

Mosaic Trisomy 6 at Chorionic Villus Sampling.

Very few examples are known. A detailed case report is given in Miller et al. (2001). A young mother had a 12-week CVS because of ultrasonographic anomalies (crown-rump length at 11 week size, nuchal translucency), with 60% of cells in short-term culture and 22% of long-term cells showing 47,XX,+6. Amniocentesis was declined. An abnormal heart rate at 25 weeks led to emergency delivery, and a growth-retarded infant with numerous anomalies was born. Her blood karyotype was normal, but trisomy 6 cells were found in placenta and umbilical cord samples. Growth indices remained below the third centile. On follow-up at age 2¾ years, neurodevelopmental progress was "near normal." Skin taken at the time of surgery showed 3% (hand) and 20% (inguinal area) mosaicism. The only two other cases on record involved mosaicism on direct preparations, followed by termination in one, and an apparently normal child subsequently born in the other.

Mosaic Trisomy 7 at Chorionic Villus Sampling.

This is typically a mitotically arising mosaicism. Kalousek et al. (1996) looked at 14 cases of trisomy 7 CVS mosaicism and fetoplacental discordance, the fraction of trisomy ranging from 7%–88% in eleven, and with three showing 100%. Twelve infants were judged normal, and in the eight of these tested, all proved to have biparental inheritance. Two infants were of low birth weight, and the one of these tested was the only of the series with UPD and a meiotic origin; the cultured CVS in this case was 100% trisomic. In a case we studied, mentioned also earlier, three postnatal placental samples karyotyped normal, and one with trisomy 7; the baby was normal (Watt et al., 1991). In the EUCROMIC study, of 32 cases with trisomy at either or both short- and long-term culture (including three with nonmosaic trisomy), none proved to have fetal involvement (Hahnemann and Vejerslev 1997). The conclusion is that the great majority of trisomy 7 mosaicism detected at CVS arises mitotically, does not imply a risk for UPD, is confined to the placenta, does not obviously compromise intrauterine growth, and is associated with the birth of a normal baby.

Mosaic Trisomy 8 at Chorionic Villus Sampling.

A well-recognized postnatal phenotype (Warkany syndrome) accompanies trisomy 8 mosaicism, which may also include an increased risk for cancer (Seghezzi et al., 1996). Fetal defects are recorded on pathology examination (Jay et al., 1999). Typically, the mosaicism is the consequence of a postzygotic nondisjunction in an initially 46,N conceptus (Danesino et al., 1998). Van Haelst et al. (2001) reviewed their experience over the period 1986–2000, based on 33,870 prenatal tests, among which were six cases of trisomy 8 mosaicism diagnosed at CVS. These six CVS cases, as it transpired, each reflected a confined placental mosaicism, and from the five pregnancies continuing a normal baby was born. A seventh case had been reported as 46,XY normal on short-term CVS culture, but the abnormal baby had mosaic trisomy 8; thus, a false-negative diagnosis. This circumstance calls to mind the scenario proposed by Wolstenholme (1996): true fetal mosaicism is typically associated with low levels of trisomy 8 in trophoblast cells (short-term CVS culture), high levels in extra-embryonic mesoderm (long-term CVS culture), and low levels in amniocytes and fetal blood cells.

Mosaic Trisomy 9 at Chorionic Villus Sampling.

Saura et al. (1995) presented seven cases of trisomy 9, five of which gave a nonmosaic result, with the outcomes being abnormal in most. In the EUCROMIC study, of nine cases with trisomy 9 at either or both short- and long-term culture (including three with nonmosaic trisomy in one or both cultures), one proved to have fetal involvement (Hahnemann and Vejerslev, 1997). This single case had nonmosaic trisomy at both short- and long-term culture. Slater et al. (2000) report a case of trisomy 9 nonmosaic at CVS, but with level II mosaicism found at amniocentesis, with only two cells 47,XX,+9. At fetal blood sampling, all 85 cells analyzed were 46,XX. Molecular studies revealed upd(9)mat. A blood sample from the newborn infant had the karyotype 47,XX,+9[4]/46,XX[50]; upon further review of the fetal blood, 3 out of 102 cells were trisomic 9. Minor anomalies were noted in the child, who had been followed up to age 1 year. It is probable that this phenotype reflected a minor degree of residual trisomy in the child's soma.

Mosaic Trisomy 10 at Chorionic Villus Sampling.

In one case, direct culture showed trisomy 10 mosaicism, while long-term culture and amniocentesis were 46,XY, but with upd(10)mat. The child subsequently born was apparently normal (Jones et al., 1995).

Mosaic Trisomy 12 at Chorionic Villus Sampling.

Hahnemann and Vejerslev (1997) and Sikkema-Raddatz et al. (1999) describe three cases, two of which involved a true fetal mosaicism. Of these latter, one fetus appeared grossly normal post termination, and one infant was abnormal.

Mosaic Trisomy 13 at Chorionic Villus Sampling.

A high level of trisomy 13 cells may well reflect significant mosaicism of the fetus. Ultrasonography and amniocentesis, and possibly fetal blood sampling, may clarify the picture. Mosaic trisomy 13 may present a very abnormal postnatal phenotype (Delatycki and Gardner, 1997). A difficulty arises in the case of very low-level (a per cent or so) mosaicism, in which case it is possible the child could be normal (Delatycki et al., 1998). In the EUCROMIC study, of 15 cases with trisomy 13 at either or both short- and long-term culture (including four with nonmosaic trisomy in one culture), two (14%) proved to have fetal involvement (Hahnemann and Vejerslev, 1997). In a series of 6820 CVS cases, Schuring-Blom et al. (2002) identified three cases of trisomy 13 mosaicism, of which two were false positives.

Mosaic Trisomy 14 at Chorionic Villus Sampling.

Only three examples of 47,+14/46,N were recorded in the EUCROMIC study, none showing fetal trisomy (Hahnemann and Vejerslev, 1997). In their case, Ralph et al. (1999) proceeded to follow-up amniocentesis, which also showed the mosaicism, and in addition maternal uniparental isodisomy 14 was demonstrated. Fetal death in utero supervened; no morphological abnormality was identified. Other prenatal cases (or retrospectively diagnosed, on postnatal placental biopsy) with the syndrome of maternal UPD14, following “correction” of trisomy, are known (Morichon-Delvallez et al., 1994; Towner et al., 2001; Engel and Antonarakis, 2002). Growth restriction, and possibly dysmorphism and minor anomalies, may be associated.

Mosaic Trisomy 15 at Chorionic Villus Sampling.

In two EUCROMIC studies, cases of trisomy 15 CPM were examined, in which direct and long-term cultures had been done (European collaborative research on mosaicism in CVS [EUCROMIC] 1999; Hahnemann and Vejerslev, 1997). Few of these cases demonstrated true fetal mosaicism. Most often, the trisomy 15, mosaic or nonmosaic, was found in cytotrophoblast and villus mesenchyme, and rarely in the fetus. The authors theorize that chromosome 15 (and 16) participates more often in trisomy rescue. This would increase the potential risk for upd15, and more often than not, the trisomy 15 would be meiotic in origin. The recommendation is that amniocentesis be offered to all patients with a CVS diagnosis of mosaic or full trisomy 15, prudently to check for the possibilities of UPD, and true fetal mosaicism. Redaelli et al. (2005) propose that the CPM might of itself lead to a phenotype, from their study of a case of severe IUGR and trisomy 15 CPM. At birth, mosaic trisomy 15 (84%) was shown by FISH on placental biopsy (which one could regard as equivalent to a CVS). The child had multiple malformations, including heart, gut, and genital, and an abnormal thymus. Postnatal chromosome analyses of blood and skin fibroblasts were normal, as had been an amniocentesis; upd15 was excluded. The child died at 6 months of age.

Mosaic Trisomy 16 at Chorionic Villus Sampling.

Almost all CPM for trisomy 16 (which may present as mosaic or nonmosaic trisomy 16 on CVS) is due to a maternal meiosis I nondisjunction. The important follow-up investigation is an amniocentesis. If this gives a normal karyotype, CPM is very probable. IUGR with a low birth weight is common, but catch-up growth is typically observed. Malformation may be present, but usually these are minor or surgically reparable birth defects. Normal intellectual capacity is well recorded (Langlois et al., 2006; Neiswanger et al., 2006). However, a more severe phenotype may result, and ultrasonography may indicate this likelihood, the complications including major malformation, and fetal death in utero. The degree of severity may relate to the presence or absence of fetal trisomy (which may not be revealed until post-natal tissue sampling), or, in the case of CPM, to the existence of uniparental or biparental disomy of the fetus, although this latter point is controversial (DeLozier-Blanchet, 2002; Eggermann et al., 2004; Langlois et al., 2006; Neiswanger et al., 2006). If trisomy 16 mosaicism is seen at amniocentesis, the prognosis is less favorable: see later.

Mosaic Trisomy 18 at Chorionic Villus Sampling.

In the EUCROMIC study, of 29 cases with trisomy 18 at either or both short- and long-term culture (including eight with nonmosaic trisomy in one or both cultures), four (14%) proved to have fetal involvement (Hahnemann and Vejerslev, 1997). Harrison et al. (1993) studied placental karyotypes from pregnancies in which trisomy 18 had been diagnosed, whether at pre- or postnatal diagnosis, and mosaicism was detected in 7 of 12, involving the cytotrophoblast. This supports the view that mosaic trisomy 18 at CVS may on occasion reflect a full trisomy of the fetus (and also leads to the conclusion that fetal survival may, in the context of this particular trisomy, be enhanced if there is a

diploid placental fraction).

Mosaic Trisomy 20 at Chorionic Villus Sampling.

Mosaic trisomy 20 is one of the commonest mosaicisms detected at amniocentesis (see later), but observation at CVS is less frequent. In the EUCROMIC study, of 12 cases with trisomy 20 at either short-term, or at both short- and long-term culture (including four with nonmosaic trisomy in short-term culture), one (8%) proved to have fetal involvement (Hahnemann and Vejerslev, 1997). Six cases were reported by Robinson et al. (2005), two of which had compromised outcomes: developmental delay in one, and growth retardation and stillbirth in the other; follow-up amniocentesis had shown trisomy at levels of 11% and 59%, respectively. Steinberg Warren et al. (2001) described a child, followed to age 8¾, normal other than hypomelanosis of Ito, from a pregnancy with a nonmosaic trisomy 20 diagnosed at CVS; culture from a subsequent amniocentesis failed. As the pigmentary skin sign in the child indicated, he was in fact mosaic, and proven to be so on skin culture; and this mosaicism would probably have been revealed, had the amniocentesis been successful. We may presume the likely circumstance as depicted in “Fetal mosaicism, nonmosaic placenta” in Figure **27–4**.

Mosaic Trisomy 21 at Chorionic Villus Sampling.

Chromosome 21 naturally commands special attention. In the EUCROMIC study, of 22 cases with trisomy 21 at either or both short- and long-term culture (including eight with nonmosaic trisomy in one culture), nine (40%) proved to have fetal involvement (Hahnemann and Vejerslev, 1997). Beverstock et al. (1998) report a “near false-negative” finding of mosaic trisomy 21, in which trisomic cells were observed in long-term CVS culture, and then, at follow-up amniocentesis, in only one culture. True mosaic trisomy was proven at fetal blood sampling, and tissue culture post abortion.

Mosaic Trisomy 22 at Chorionic Villus Sampling.

Fetal defect is typically associated, but the degree may vary considerably. Wolstenholme et al. (2001a) described their own case of nonmosaic trisomy 22 diagnosed at direct and cultured CVS, with 47,XX,+22/46,XX mosaicism subsequently shown at amniocentesis (3/60 cells +22) and fetal skin biopsy (6/170 cells +22). Fairly subtle fetal dysmorphism was noted post termination, and multiple tissue samplings showed mostly low but consistent trisomy mosaicism: 1% trisomic cells in skin, muscle, blood, kidney, 3% in lung, 5% in liver, and 21% in spinal cord. It is probable that neurological compromise would have transpired, quite likely of severe degree, had the child been born. Wolstenholme et al. reviewed 11 other cases of mosaic and nonmosaic trisomy 22, the mosaicisms mostly being of high percentages at CVS, and (in the six cases proceeding to amniocentesis) low percentages at amniocentesis. Of nine cases in which posttermination samplings were done, six showed mosaicism in at least some tissues (see also the case of De Pater et al., 1997, mentioned earlier in the section on “Level III Mosaicism”). In the three cases with 0% trisomy at fetal sampling, all had manifested severe intrauterine growth retardation. This may have been the consequence of functional insufficiency of the trisomic 22 placenta; there is also the point that occult fetal trisomy can never be excluded. Bryan et al. (2002) studied a child born of a pregnancy with a nonmosaic 47,XY,+22 karyotype having been shown at CVS. There was IUGR, but the child apparently showed postnatal catchup. He typed 46,XY on peripheral blood (with biparental disomy) and was phenotypically normal, except for hypospadias.

Detection at Amniotic Fluid Cell Culture

Considering the three major trisomies, Hsu et al. (1992) have determined that mosaicism for chromosomes 13, 18, and 21 very frequently predicts fetal abnormality, in half or more of cases. As for rare trisomies, Hsu et al. (1997) have undertaken a wide survey, based on the experiences of a number of American and Canadian laboratories and drawing on previous reports in the literature; the reader wishing full detail will need to refer to the original document. Some mosaic trisomies are associated with a high risk for phenotypic abnormality in the fetus or term infant, with figures of >60% for mosaic trisomies 2, 16, and 22, while trisomies 7, 8, and 17 are toward the lower end of the scale (<20%). Ultrasonography has a role in the assessment: most cases in which the mosaicism involves the fetus to a substantial degree will display morphologic/growth abnormality. Nevertheless, normal ultrasonography cannot allow firm reassurance. Some mosaic states might cause structural defects too subtle to be discerned at fetal imaging, and yet be associated in the child with considerable, possibly severe functional neurological compromise. In chromosomes known to be subject to parent-of-origin imprinting, uniparental disomy needs also to be factored in to the assessment. Comments on individual trisomies follow.

These are rare observations, and in the survey of Forabosco et al. (2009), the most frequent mosaic autosomal trisomies recognized at amniocentesis were, in descending order: trisomies 21 (1 in 4000 amniocenteses), 20 (1 in 5000), 13 and 18 (1 in 22,000), 9 (1 in 30,000), and, each at 1 in 90,000, trisomies 2, 6, 7, 8, 15, and 17.

Mosaic Trisomy 2 at Amniocentesis.

In Hsu et al.'s (1997) survey, trisomy 2 conveyed the highest risk of any of the “rare trisomic” autosomes for an abnormal outcome, namely 90%, with a variable pattern of major defects. It is probable that mosaic trisomy 2 detected at amniocentesis would be in the same group as the high-level mosaic CVS case (see earlier). A trisomic line in the fetus/child may take some diligence to find. Sago et al. (1997) reported a case in which there was level III mosaicism with trisomy 2 cells present in 27% of amniocytes (and biparental disomy). The child was severely abnormal, and while blood and skin karyotyped as 46,XY, 4% of liver cells were 47,+2.

Mosaic Trisomy 3 at Amniocentesis.

Only two cases were identified in Hsu et al.'s (1997) review, in one of which the child had multiple malformations, with the mosaicism confirmed on skin fibroblast culture. The child in the other case was normal. Marked intrauterine, and subsequently postnatal growth restriction, was the prominent feature in the cases in Zaslav et al. (2004) and Sheath et al. (2010); in both, development in early infancy was judged to be normal.

Mosaic Trisomy 4 at Amniocentesis.

A very few cases have been reported, with normal and abnormal outcomes both, approximately proportional to the fraction of trisomic cells (see trisomy 4 in CVS earlier; Marion et al., 1990; Zaslav et al., 2000; Chen et al., 2004a; Gentile et al., 2005). A single-digit percentage of +4 cells has been associated with normality, on follow-up to 1 year of age. Likewise, normal ultrasonography is a positive pointer.

Mosaic Trisomy 5 at Amniocentesis.

Hsu et al. (1997) recorded five cases. In one, a high level of trisomic cells (80%) was associated nevertheless with a phenotypically and karyotypically normal infant. In two, the child was abnormal, both showing the mosaicism on postnatal study. Brown et al. (2009) identified 50% mosaicism in a pregnancy in which an ultrasonographic heart abnormality had been seen, and subsequently terminated at 21 weeks. A dysmorphic facial appearance was noted, and the cardiac defect confirmed, at fetal pathology. The trisomy was confirmed in fetal tissues (excepting blood), although at a lesser level of mosaicism that that of the amniocyte culture.

Mosaic Trisomy 6 at Amniocentesis.

Hsu et al. (1997) recorded three cases, each with the same low-level (6%) trisomy in amniocytes, and each with a normal outcome. Reports exist of the diagnosis following recognition of fetal defects at ultrasonography, the defects ranging from minor to severe (Wallerstein et al., 2002; Wegner et al., 2004; Destree et al., 2005). One case of fetal death in utero at 23 weeks was associated with 48% trisomy cells on fetal skin analysis (Cockwell et al., 2006). Chen et al. (2006b) report a case with low-level (3%–10%) mosaicism, with normal fetal blood karyotype, biparental inheritance, in which the parents chose termination, and the trisomy was absent on cultured fetal tissue. They suggest low-level trisomy 6 mosaicism may be a benign finding.

Mosaic Trisomy 7 at Amniocentesis.

Hsu et al. (1997) recorded eight cases, with fractions of trisomic cells ranging from 5% to 48%. Only one resulted in the birth of a phenotypically abnormal child, but low-level

47,XY,+7/46,XY mosaicism was confirmed in two phenotypically normal children on foreskin analysis. Warburton (2002) emphasizes that this relatively low-risk assessment is the appropriate one to offer, and she notes also that UPD7, while unlikely, may be worth testing for. A low-level mosaic case, taken to termination with pathology study and multiple fetal karyotyping, with entirely normal findings, led these authors (Chen et al., 2005b) to agree with the view that optimistic advice may usually be appropriate.

Other cases with abnormal outcome (and in which ascertainment was necessarily biased) include the following. Mosaicism was verified postnatally on skin fibroblast analysis in the child reported in Kivirikko et al. (2002), in whom fetal blood sampling and midtrimester ultrasonography had been normal; there was facial asymmetry and mild dysmorphism along with rather impressive hypomelanosis of Ito, while mental development was "considered to be within normal limits," although no detailed assessment had been done. The fraction of trisomic colonies in the 47,XX,+7/46,XX case of Bilimoria and Rothenberg (2003) was rather high, at 41%, and in addition uniparental heterodisomy was shown in the 46,XX line; the pregnancy had come to attention because of an increased-risk maternal serum screen. On a neonatal blood sample, all cells were 46,XX, while on the contrary all placental cells analyzed were trisomic. The child was small for dates and had some minor anomalies. These authors mention an anecdote of a trisomy 7 mosaic woman "graduating from college and getting married." Petit et al. (2011) describe a case of intrauterine growth retardation leading to amniocentesis, which was interpreted at the time as normal. The child proved to be retarded in growth and development, and displayed hypomelanosis of Ito. Blood analysis showed 46,XY with maternal UPD 7. The skin fibroblast karyotype, however, was 47,XY,+7/46, XY; and restudy of stored amniocytes from long-term culture showed mosaic trisomy 7. These authors provide a useful review.

Mosaic Trisomy 8 at Amniocentesis.

Counseling is difficult, and advice must be cautious. An observation of trisomy 8 in amniocytes predicts a distinct probability, but by no means a certainty, of the clinical syndrome. It is not possible to put a good figure on the level of risk. Vice versa, a true fetal mosaicism may not necessarily be detected at amniocentesis (Wolstenholme, 1996). A finding of apparently normal morphology at fetal examination following termination in some 47,+8/46,N pregnancies might be misleading, since the physical component of the clinical syndrome is relatively minor (Hsu et al., 1997). In the series of van Haelst et al. (2001) mentioned earlier, the two cases of trisomy 8 mosaicism detected at amniocentesis both turned out to be pseudomosaicism.

Mosaic Trisomy 9 at Amniocentesis.

The risk is high (Saura et al., 1995; Chen et al., 2003a). Hsu et al. (1997) recorded data on 25 cases, with pregnancy termination being done in 21. Abnormality was identified in most of the 21, and mosaicism confirmed in the seven having skin fibroblast studies. In the four pregnancies continuing, one abnormal child was born, with 47,+9/46,N mosaicism on blood karyotyping, the other three pregnancies resulting in apparently normal newborns. An overall figure of 56% applies for the risk that the fetus is abnormal. This high percentage figure is not surprising, and indeed it may well be an underestimate of the risk for functional abnormality in the child (intellect not being assessable in the newborn), considering the well-recorded phenotype of mosaic trisomy 9 in older individuals. A review of surviving individuals whose parents participated in a survey is given in Bruns (2011).

Mosaic Trisomy 10 at Amniocentesis.

In one case listed in Daniel et al. (2004), at amniocentesis a 47,XX,+10[27]/46,XX[83] karyotype was associated with severe fetal defects, this observation being the basis of the referral for prenatal diagnosis. They were able to ascertain that the cause was a postzygotic duplication of the maternal homolog.

Mosaic Trisomy 11 at Amniocentesis.

Of the four reported examples, all have had a normal outcome. One child came from a pregnancy with a 26% fraction of trisomic cells, with 46,N findings on postnatal tissues, and followed through to 1 year of age. Basel-Vanagaite et al. (2006) raise the question that this mosaicism may typically be a benign finding.

Mosaic Trisomy 12 at Amniocentesis.

This is one of the more frequently described mosaicisms, and often implies a high risk. Hsu et al. (1997) accumulated 23 cases, comprising 12 continuing pregnancies and 11 terminations. In most of those proceeding to fetal or neonatal fibroblast karyotyping, the mosaicism was subsequently confirmed, albeit that most of the fetuses appeared to be normal. It is possible, however, that some subtle physical features, and possibly unsubtle neurological deficit, might have eventuated had these "normal" fetuses been born. The clinical range in the few recorded liveborn patients with true trisomy 12 mosaicism is very variable, from lethality in the newborn period, through to an otherwise normal man with Kartagener syndrome being investigated for infertility (DeLozier-Blanchet et al., 2000). Of the 12 continuing pregnancies in Hsu et al. the outcomes were abnormal in 5, and grossly normal newborns in 7. Three of these normal infants followed for 5 months to 5 years were all judged to be continuing to be normal, and Staals et al. (2003) add another 3-year-old to this list. The proportion of trisomic cells at amniocentesis apparently is not a very helpful guide in prognosis. In one case in Daniel et al. (2004), associated with fetal defect at 18-week termination, the trisomy had resulted from a postzygotic duplication of one homolog.

Mosaic Trisomy 13 at Amniocentesis.

The risk for abnormality is very high. A collaboration of 23 American and Canadian laboratories provided data on the outcomes of 25 prenatal diagnoses of 47,+13/46 mosaicism (Wallerstein et al., 2000). Care was taken to exclude cases in which ascertainment had been biased by abnormal ultrasonography. In 21, the pregnancies were terminated. Various abnormalities were identified in 10 of these; the range of percentages of abnormal amniocytes was very wide, 6%–94%, average 58%. No defect was detectable in the remaining 11 aborted fetuses, although the assessment was limited to simple inspection. Four pregnancies proceeded to apparently normal livebirth; the percentages of abnormal amniocytes in these were lower, ranging 5%–13%.

Mosaic Trisomy 14 at Amniocentesis.

Hsu et al. (1997) recorded data on five cases. In the three choosing to continue the pregnancy to term, the infants appeared normal, and typed 46,N. In the two opting for termination, fetal abnormality was shown, in one case comprising hydrocephaly. A risk exists for UPD 14, over and above any defect due to the mosaic trisomy per se, and this should be checked (see also earlier section on "Mosaic Trisomy 14 at Chorionic Villus Sampling").

Mosaic Trisomy 15 at Amniocentesis.

Trisomy 15 is usually the consequence of a maternal meiosis I nondisjunction. Amniotic fluid mosaicism may well reflect a true mosaicism of the fetus. In Hsu et al. (1997), six of the eleven cases recorded had an abnormal outcome, the risk being greater when the trisomy level was higher (>40%). Zaslav et al. (1998) review seven cases of low-level mosaic trisomy 15 detected at prenatal diagnosis, in each the amniocentesis having been done for advanced maternal age. All seven chose to terminate, and a variety of defects were documented in most but not all. In their own case, the trisomic cell line in the initial amniocyte analysis was at a low level: 47,XX,+15[2]/ 46,XX[37]. Fetal tissues were also at low levels (lung 2%–5%, heart 8%–15%, skin 6%–10%, on metaphase and interphase analysis, respectively), but the placenta showed 100% trisomy on metaphase analysis and 95% using FISH on interphase cells. These authors also document from the literature four cases of abnormal liveborns with trisomy 15 mosaicism. There is the additional question of upd(15)mat, the considerable phenotypic consequence of which—namely, Prader-Willi syndrome—may be superadded upon that of a trisomy 15 mosaicism.

Mosaic Trisomy 16 at Amniocentesis.

Neiswanger et al. (2006) conducted an exhaustive literature review of trisomy 16 mosaicism diagnosed prenatally, including 36 cases from amniocentesis; and they reported their own findings in three cases in which no prior CVS had been undertaken. Of these three, all had abnormal outcomes: IUGR but with normal cognitive development as judged at 14 months; IUGR and major malformations including cardiac dextroposition; and IUGR with hypoplastic left heart, leading to neonatal death. In their literature review,

the figures for complication were as follows: infant death, 33%; prematurity, 64%; IUGR, 69%; physical anomalies, 75%; and just one assessed as a normal outcome, 3% (these figures being considerably worse than for CVS diagnosis). They note that level II mosaicism, in this context, may well reflect a true fetal mosaicism. The presence of UPD appeared not to influence the rates of prematurity or infant death; however, UPD was more frequent in those pregnancies with IUGR or infants with anomalies. Yong et al. (2003) tested for UPD in a series of infants from mosaic trisomy 16 pregnancies, and the fraction with upd(16)mat, at 40%, was close enough to the one-third expectation from random loss of one chromosome; and these infants were more severely affected than those with biparental inheritance of 16. Mosaic trisomy 16 also carries an associated risk for the mother of preeclampsia (Yong et al., 2006). Thus, the earlier opinion of Hsu et al. (1998) is supported: “mosaic trisomy 16 detected through amniocentesis is not a benign finding but associated with a high risk of abnormal outcome, most commonly intrauterine growth retardation, congenital heart defect, developmental delay, and minor anomalies.” Rieubland et al. (2009) diagnosed two cases postnatally, noting a considerable phenotypic difference between the two, one normally grown and developing at age 11 months, but with a severe hypospadias, the other with IUGR, body asymmetry, numerous physical anomalies, and dying at 7 months; yet further illustrating the challenge in offering advice at prenatal detection. Notwithstanding, we have seen an eventual normal outcome, the child assessed at 2½ years of age, albeit delivery by cesarean section at 36 weeks had been necessitated due to fetal distress with IUGR. Trisomy 16 had been detected at high level on CVS and at amniocentesis, and low-level (8%) postnatally on buccal mucosal cells (Coman et al., 2010).

Mosaic Trisomy 17 at Amniocentesis.

This trisomy vexes, with normal and abnormal outcomes equally observed. Hsu et al. (1997) comment that the diagnosis “should not be taken lightly,” mirrored by Utermann et al. (2006) who state that “the clinical significance remains uncertain.” The longest follow-up is reported in Witters and Fryns (2008), a child at age 36 months, who was significantly delayed, with a developmental age of 26 months. And yet a number of normal outcomes are on record, as Abrams et al. (2005) document in their own case, with the child reportedly normal as a 2-year-old, and as they note similarly in a handful of other cases from the literature. They advise that an optimistic view is warranted, if the ultrasonography is normal. The cerebellum should be targeted, since malformation may be a feature of trisomy 17.

Mosaic Trisomy 18 at Amniocentesis.

The risk is very high. In the collaboration of Wallerstein et al. (2000), 31 prenatal diagnoses of trisomy 18 mosaicism were available for review. In just over half of these, the abortuses (induced termination or natural abortion) were abnormal. In 11, no defects were discerned at fetal examination. Just three pregnancies came to live birth, and these babies were apparently normal. The percentages of trisomic amniocytes in these three cases ranged from 2% to 20% (mean 9%), compared with 2%–95% (mean 37%) in those with abnormal outcome. A very rare, but recurrent abnormality is 45,X/47,XX,+18 mosaicism, in which the phenotype can vary from fairly mild to severe (Schluth-Bolard et al., 2009; Tyler et al., 2009).

Mosaic Trisomy 19 at Amniocentesis.

A single case is recorded in Hsu et al. (1997), and in which there was a normal outcome at live birth.

Mosaic Trisomy 20 at Amniocentesis.

This is one of the most commonly observed mosaic aneuploidies. Trisomy 20 may exist in three forms: as confined placental mosaicism, as placental-fetal mosaicism with an apparently normal phenotype in the child that is subsequently born, or as a fetal mosaicism with phenotypic consequence (Hsu et al., 1991). There may be no dysmorphic features, or only some “soft” signs, or rarely an unambiguous facial dysmorphism; a characteristic, if subtle syndrome is proposed (Willis et al., 2008). In certain fetal regions in which the trisomy may exist, in particular kidney and gut, the imbalance apparently has no discernible untoward effect, and in fact aneuploid cells may be cultured from urinary sediment. (Recognizing that amniotic fluid has a substantial contribution from fetal urine production, presumably some of the “amniotic fluid cells” from which the diagnosis of trisomy 20 had been made may have actually had origin from the fetal urinary tract.)

In the collaboration of Wallerstein et al. (2000) comprising 152 diagnoses, ten (7%) were recorded with an abnormal outcome (six liveborns, four abortuses). There was correlation with the level of mosaicism: abnormality was observed in 20% of infants where there had been >50% trisomic cells at amniocentesis, and in 5% of those with <50%. Baty et al. (2001) reviewed 17 cases in which follow-up of the children extended beyond 1 year, of whom 12 (71%) had developed normally. The remaining five had various degrees of speech and motor delay. A more optimistic interpretation comes from James et al. (2002), who tracked down all cases diagnosed at amniocentesis in New Zealand 1991–2001, numbering 13, with follow-up well into childhood for 9 of these (the longest to age 10). The range of the trisomic fraction of amniocytes was 8%–50%. All were essentially normal, except for one child who had minor anomalies at birth, resolving by 6 months of age, and deformation due to breech delivery may have been the cause, although weight was below the 3rd centile; and in the only case in which termination was chosen, rather subtle (indeed borderline) external fetal anomalies were noted, and cultured tissue showed low-level (skin 2%, kidney 7%) trisomy mosaicism. Baty et al. (2001) followed up two cases with higher fractions of trisomic cells at amniocentesis, 83% and 57% in one, and of 90% in the other, and the children, at ages 9 and 8 years, respectively, were of normal intelligence and of essentially normal morphological appearance. Each did, however, display quite prominent hypomelanosis of Ito, presumably reflecting a fairly widespread distribution of a trisomic 20 lineage, at least in skin.

Nevertheless, reservation must remain. Reish et al. (1998) offer the sobering example of a 15-month-old child with considerably delayed gross and fine motor skills and poor language acquisition, who had 54% trisomic cells from a skin biopsy (a normal karyotype on peripheral blood). In the pregnancy, amniocentesis had shown a 45% mosaicism, fetal ultrasonography was normal, and the parents had been “cautiously counseled.” Likewise, Wallerstein et al. (2005) report a child who had seemed normal at birth, and 46,XX on blood, but who went on to manifest a “pervasive developmental disorder.” Trisomy 20 had been present in only 4/63 colonies at amniocentesis; trisomy was further documented in urinary sediment at age 4 years. They comment that “optimism regarding developmental outcome should be tempered with some caution.”

Bianca et al. (2008) summarize the issues and advise along these lines: a second CVS or amniocentesis would add little value; fetal blood sampling is not useful, and neither is UPD analysis; the level of mosaicism does not predict outcome (this agrees with the views of some, and contradicts others, as noted earlier); and some reassurance may be gained from normal ultrasonography.

Mosaic Trisomy 21 at Amniocentesis.

The risk for Down syndrome is very high. The collaborative study of Wallerstein et al. (2000) accumulated 96 cases for review. Half had an observably abnormal outcome, with confirmatory cytogenetic study performed in a minority. Most of these were fetuses post termination with various abnormalities; six were liveborns, five of these having a clinical diagnosis of DS, and one an isolated heart defect. An apparently normal appearance (assessment limited to inspection in 39, autopsy in 2) was recorded in 41 aborted fetuses. Among these, 20 were submitted to further cytogenetic analysis (repeat amniocentesis, fetal tissue, fetal blood, placenta), with 8 showing 8%–90% trisomic cells, and 12 with 0%. Seven liveborns were normal, two being followed up beyond the newborn period; none had confirmatory karyotyping. The mean amniotic fluid proportion of trisomic cells was 17%, range 6%–31%, in these normal children. This compares with a mean of 35% in those with a demonstrably abnormal outcome. But even in the group with the lowest level of amniotic fluid trisomy, 3%–10%, half had an abnormal outcome. From the whole material, a risk for phenotypic abnormality of 50% should be seen as a minimum estimate, since subtler defects at fetal or neonatal assessment would have escaped notice, and a potential compromise of intellectual function of course was not assessable. There is a maternal age association with this mosaicism (Forabosco et al., 2009).

Mosaic Trisomy 22 at Amniocentesis.

Hsu et al. (1997) determined a very high risk for abnormality for 47,+22/46, with 7 out of 11 outcomes being abnormal. Berghella et al. (1998) described a normal fetal blood result following trisomy 22 mosaicism diagnosis at amniocentesis, but fetal skin biopsy showed 47,+22/46, and structural abnormalities were subsequently identified in the aborted fetus. Four cases are noted in the review of Wolstenholme et al. (2001a), these all having followed an initial detection at CVS. Three out of the four showed some degree of normal/trisomy mosaicism at fetal samplings post termination. Leclercq et al. (2010) record a normal phenotypic outcome in a single case, followed up to age 4 years, albeit that the child showed the mosaicism on skin, in 6% of cells. Three other cases were abnormal at autopsy study (two following fetal death in utero, and one a medical

termination).

Mosaic Partial Trisomy at Amniocentesis.

It is not feasible to list here recorded cases, and each must be judged on its merits. One specific example is worth noting, in that it may represent simply cultural artifact associated with a fragile site. This is mosaicism for a del(10)(q23). Zaslav et al. (2002) document a case of 46,XY,del(10)(q23)[9]/46,XY[45] detected at amniocentesis. The phenotypically normal child had the del(10q) in only 3/100 blood cells, this culture having been stressed by growth in a low-folate medium. We are aware of a handful of essentially similar case, all involving 10q23, and none resulting in a documented abnormal child. The biology here is uncertain, as amniotic fluid is normally cultured under conditions that suppress fragile site expression. Indeed it is not clear that the known fragile site FRA10A at 10q23 (p. 261) is actually involved.

Polyploidy

Triploidy.

Close to 100% of the time, triploidy aborts spontaneously, but in some cases not until the pregnancy is well advanced. This being so, the offer of termination is appropriate when triploidy is diagnosed. Cassidy et al. (1977) described the emotional turmoil suffered by the family when a triploid infant, predicted to die immediately, survived for the extraordinary period of 5 months. Sarno et al. (1993) reported a unique case of complete placental/fetal discordance with triploidy on CVS and a normal diploid karyotype on amniocentesis and fetal blood sampling, with the birth of a normal baby; such a possibility warrants consideration where triploidy on CVS accompanies an ultrasonographically normal fetus. Nonmosaic triploidy typically shows ultrasonographic anomalies, and according to the diandric (partial mole) or digynic (asymmetric IUGR) nature of the imbalance (p. 287).

True triploidy mosaicism is very rare (p. 289). Wegner et al. (2009) report a prenatal diagnosis, the pregnancy ending in fetal death in utero at 25 weeks, with the remarkable mixed-gender karyotype of 46,XX/69,XXY. Numerous abnormalities were revealed at anatomical pathological examination. They were able to demonstrate that the initial conception had been dispermic (one X and one Y-bearing sperm), and that the 69,XXY lineage had arisen by the delayed incorporation of the Y-bearing male pronucleus into a cell with a 46,XX nucleus; they preferred the expression “mixoploidy” to describe this scenario.

A very rare case is “hypotriploidy” with 68 chromosomes. One case of 68,XX hypotriploidy was diagnosed prenatally following an ultrasound picture which was similar to that of classic digynic triploidy (Pasquini et al., 2010).

Tetraploidy.

Tetraploidy seen at prenatal diagnosis, in the context of normal ultrasonography, is usually an in vitro cultural artifact, or possibly a vestige from the blastocystic stage of normally occurring trophoblastic tetraploidy (Krieg et al., 2009). True tetraploidy is very rare, and Teyssier et al. (1997) record only ten cases, two of which had been discovered at amniocentesis. Ultrasonographic demonstration of growth retardation and enlarged cerebral ventricles may be typical, but rather nonspecific signs. While tetraploid/diploid mosaicism is almost always a cultural artifact, Edwards et al. (1994), having observed true normal/tetraploid mosaicism in two severely retarded individuals, nevertheless caution that a tetraploid cell line is not absolutely certain to be an innocuous finding. In a single such case at prenatal diagnosis, Meiner et al. (1998) showed 92,XXYY/46,XY mosaicism on fetal blood sampling following the diagnosis of nonmosaic 92,XXYY at amniocentesis, in the setting of growth retardation discovered at ultrasonography and confirmed at subsequent fetal pathology study.

Structural Rearrangement

Structural rearrangements are seen in about 1 in 1000 cytogenetic prenatal diagnoses (Warburton, 1991). It is typically a matter of urgency to do parental chromosome studies, in order to distinguish between a familial or a de novo rearrangement in the fetus. If one parent is discovered to have the same apparently balanced autosomal rearrangement identified at prenatal diagnosis, and in the context of normal ultrasonographic anatomy, there is no firm evidence for an increased risk of fetal abnormality, and many would counsel to the effect of no discernibly increased risk. Sex chromosome rearrangements require separate attention.

De Novo “Apparently Balanced” Structural Rearrangement

A major difficulty is posed by the de novo rearrangement that, at the level of classical cytogenetic analysis, is “apparently balanced,” and when the interpretation at ultrasonography is normal. But even with the highest resolution banding, a submicroscopic abnormality (deletion or duplication, or gene disruption) may still be present. It may be that the occurrence of breakpoint(s) within a G-negative band, these being more gene dense, predicts a greater risk for abnormality (Fantes et al., 2008). Microarray analysis can, in these cases, be revealing, because small deletions or duplications at the breakpoint may be identified; however, a true disruption of a gene, with no net gain or loss of DNA, would not be detected (De Gregori et al., 2007; Baptista et al., 2008). Nevertheless, we should emphasize the observation that most pregnancies with prenatal diagnosis of a de novo inversion or simple reciprocal translocation go on to produce a normal baby. Presumably, these normal cases reflect breakpoints in DNA that does not code for a gene or for a control element (or if a gene is disrupted, its haplo-state is sufficient), and in which there is no concomitant microdeletion.

Of course, abnormal ultrasonography dictates a different perspective. Thus, for example, when Price et al. (2005) identified growth and anatomical abnormalities suggestive of Cornelia de Lange syndrome (CdLS), the subsequent finding of a presumed de novo translocation (father not available for testing) 46,XX,t(3;5)(q21;p13) enabled a clear interpretation, the CdLS gene being located at 5p13, and presumably disrupted by the rearrangement.

On postnatal observation, one can be wise after the event. If a child with a particular phenotype has a rearrangement involving a breakpoint known to be in the region of a mendelian locus, or of other recorded rearrangements producing the similar phenotype, the conclusion could reasonably be drawn that the cytogenetic abnormality was the cause of that abnormal phenotype. For example, a child with a de novo inv(7)(p22q21.3) having a particular split hand/foot malformation would invite the inference of a causal link, given the similarity of the limb defect with other 7q21.3–22 rearrangements (Cobben et al., 1995). Sophisticated tools of the molecular cytogeneticist may reveal a hidden defect, such as an apparently balanced de novo 14q paracentric inversion in which Jiang et al. (2008) could actually show very small deletions at both breakpoints; these authors list the genes within the deleted segments and speculate about their possible contributions to the abnormal phenotype of the child in whom it was identified (and see also Chapter 9). In a normal person, on the other hand, an apparently balanced rearrangement we may take to be truly balanced. Caution should be exercised in the interpretation of apparently balanced translocations in which microarray testing detects an imbalance, and Gajecka et al. (2008a) provide several examples of gains and losses at the breakpoints in apparently balanced translocations in phenotypically normal individuals.

Empiric Risk Estimation.

Warburton (1991) conducted a review of major laboratories in the United States and Canada over a 10-year period, and collected data based on more than a third of a million procedures. We make frequent reference to this work. A de novo translocation was identified in about 1 in 2000 amniocenteses, a Robertsonian translocation in about 1 in 9000, and an inversion in 1 in 10,000. She emphasizes that the outcome data are imperfect, given the lack of long-term follow-up, and the questionable accuracy of phenotypic assessment in terminated pregnancies. Having made that point, she does say “there was no case in which a live birth originally reported as normal was later classified as abnormal after longer follow-up. In fact, the opposite tended to occur: several cases described as having neonatal problems were later described as completely normal.”

Small studies with follow-up into childhood have been undertaken (Gyejye et al., 2001), and these suggest that the figures presently offered are in the vicinity of the truth, but a clearer answer will require quite large numbers of children to be assessed. In an 8-year retrospective study from Taiwan, Peng et al. (2006) reviewed neonatal outcomes from

66 balanced rearrangements, but of which only 15 were de novo (reciprocal and Robertsonian translocations, inversions). If the anomaly rate in the inherited cases (2.0%) were to be taken as a baseline, the excess (4.7%) observed in the de novo cases (6.7% in total) could in theory be interpreted as the residual increased risk of anomalies due to the rearrangement; however, since the numbers in each category were so small, in fact little weight can be placed upon them.

Given the long experience with prenatal diagnosis now accumulated, it is perhaps surprising that the data are as deficient as they are; or yet, if one considers the reality of what is involved in the logistics of long-term follow-up, perhaps not. A large collaborative exercise involving 29 Italian prenatal laboratories, covering the period 1983–2006, brought together the findings on a total of somewhat more than a quarter of a million diagnoses (amniocentesis, CVS, and fetal blood) (Giardino et al., 2009). From these, 246 de novo balanced rearrangements were identified: 177 reciprocal translocations, 45 Robertsonian translocations, 17 inversions, and 7 complex chromosome rearrangements. But follow-up data, in the 80% of cases where the pregnancy was continued, were insufficient to derive risk figures for clinical outcomes, due to logistic and legal considerations, albeit that the authors comment that “none of the newborns have been reported to display visible malformations.” We hope others who might be in a position to access similar data will not be too discouraged; one can offer a note of reassurance for the researcher (and for members of ethics committees) that most parents, in the slightly different setting of having had the news of an ultrasound abnormality in pregnancy, are willing to respond to requests for information about how well their children subsequently did (Ramsay et al., 2009, and as we discuss on p. 20). Equally, it may be that microarray analysis will enable a clearer view, once our understanding of CNVs has settled down; the fine focus due to microarray might bypass the need for a risk estimate by directly recognizing a balanced, or an unbalanced, genome.

As we have commented in Chapter 4, it is good to have good figures, in order to inform a reproductive decision. But an accurate percentage figure is, of course, only one factor in the equation. Each woman or couple will come with their own viewpoint, their own psychology, and their own mix of emotions. Wallerstein et al. (2006), in a retrospective survey of genetic counselors in North America, determined that the degree to which the news of a de novo rearrangement caused anxiety is a key factor in leading to a decision to continue the pregnancy or to terminate.

The “Carrier Fetus” Who Will Become a Carrier Adult.

We have discussed in the introductory chapter the issue of the genetic testing of children. In the case of prenatal diagnosis in which a de novo apparently balanced state is discovered, of course the child has already been tested, and “untesting” not a practical matter. Consider the example of the mosaic test result mentioned earlier, the whole-arm translocation 46,XY,t(1;5)(p10;q10)/46,XY. Naturally, parents may want to know what reproductive implications this may have for their as-yet-unborn child. In this example, the genetic risk for the child will be, as the reader can readily determine, essentially that of a likely propensity to miscarriage, should the translocation cell line involve the gonad. It is the counselor's responsibility to communicate this sort of information in outline form to the parents, along with the advice that the child could, in the fullness of time, attend the clinic on his or her own behalf. The information must be clearly conveyed. It could be seen as a failure of the counselor's duty of care if, in the next generation, an affected child were born, the parents being unaware of the genetic risk (Burn et al., 1983).

De Novo Balanced Reciprocal Translocation

Simple Translocation.

The starting point is that precedents are recorded for a de novo translocation having disrupted or compromised a locus, and therefore that the discovery of such a rearrangement at prenatal diagnosis could potentially herald an abnormal child. Of course, these translocations are to be taken seriously. Equally, the balanced carrier state (every one of which in the world must have been de novo at some point in the near or distant past) is very familiar, as Chapter 5 attests at length. Very many translocations are truly balanced, in terms of their functional genetic consequences. Thus, a normal child is very possible, and as the observations have shown, this is considerably the more likely outcome. In Warburton's study, serious malformations were identified in 6% of pregnancies with a de novo simple reciprocal translocation, either at elective termination or at live birth. This is some 3% above the background risk of around 3% for malformation and/or serious functional defect that applies to all pregnancies. Thus, we may draw the inference that in about 3% of these de novo translocations the chromosomal defect was causative. It seems reasonable to assume that a slightly higher figure, perhaps another percent or so, should apply to the overall risk for not only major malformation but also important functional deficit, which might not become apparent until after babyhood. Normal ultrasonography would be somewhat, but not definitively reassuring.

As mentioned earlier, microarray studies may well cast light. In a study of 14 prenatal diagnoses of de novo simple translocation, the ultrasonography being normal in 12, all proved to be balanced at the level of array-CGH (De Gregori et al., 2007). This type of study provides a springboard for interpretation when a de novo translocation is encountered at prenatal diagnosis, and the option of microarray is available.[7]

Whole-Arm Translocation.

Very few de novo whole-arm translocations are recorded, “although the existing examples suggest an optimistic prognosis can be given” (Farrell and Fan, 1995). A whole-arm X-autosome translocation is mentioned later.

Complex Rearrangement.

A de novo apparently balanced complex chromosome rearrangement (CCR) has a high risk for intellectual impairment and physical malformation, but equally, completely normal children have been born. Chen et al. (2006a) and Giardino et al. (2006) reviewed the published cases, in some of which amniocentesis had been triggered by an increased-risk maternal serum screen, or the observation of fetal anomaly on ultrasound. The outcomes were abnormal in around a half, the abnormalities ranging from developmental delay, through single or multiple malformation. Intuitively, the risk would be greater with a higher number of breakpoints, and Madan et al. (1997) provide support for this view. Microarray analysis may clarify whether a true quantitative imbalance exists; however, a CCR with a breakpoint occurring within a gene might (as with any such rearrangement) not be detected, as exemplified in the t(2;12;18)(q22.3;12q22;q21.33) reported in Engenheiro et al. (2008), in which the 2q22.3 breakpoint disrupted the *ZEB2* gene, causing Mowat-Wilson syndrome. In a report of three CCRs diagnosed prenatally, all proved to be unbalanced upon array-CGH analysis (Dr Gregori et al., 2007).

Mosaicism for a De Novo Structural Rearrangement in Balanced State

Reciprocal Translocation Mosaicism.

True mosaicism for a balanced reciprocal translocation, 46,rcp/46, is very rarely recognized (Fryns and Kleczkowska, 1986; Opheim et al., 1995; Leegte et al., 1998). The great majority of this type of mosaicism seen at prenatal diagnosis is level I or II and is pseudomosaicism due to in vitro change. Some breakpoints (6p21, 13q14) are preferentially involved (Benn and Hsu, 1986). In terms of implications for fetal phenotype, it can usually be disregarded. True mosaicism for a reciprocal translocation has been reported at prenatal diagnosis, and Hsu et al. (1996) accumulated eleven examples showing one normal cell line and one with a balanced autosomal translocation. In no instance in which the pregnancy proceeded (nine of the eleven) had phenotypic abnormality been observed. Concerning a possible risk for unbalanced progeny in the next generation if the gonad were involved, each such case would need to be individually assessed; the parents would need to know to give their child access to the information in due course.

Robertsonian Translocation Mosaicism.

In four cases in Hsu et al. (1996) of diagnosis at amniocentesis of mosaicism for a balanced heterologous translocation, 45,rob/46, the outcome was normal in all (the mosaicism confirmed postnatally in the two infants studied). The specific translocations were 13q14q, 13q22q, and 14q21q.

Whole-Arm Translocation Mosaicism.

The mother reported in Wang et al. (1998) with 46,XX,t (10q;16q)/46,XX mosaicism was normal (although her child abnormal; see p. [link]). We know of one case of level III mosaicism for a whole-arm translocation at amniocentesis, 46,XY,t(1;5)(p10;q10)/ 46,XY, with 30% of cells in three separate cultures showing the translocation, and confirmed on a cord blood sample at delivery (10 cells out of 50 with the translocation); on follow-up at age 4 years the child was normal and healthy (personal communication, D. Grimaldi and B. Richards, 2001).

Complex Rearrangement Mosaicism.

The only known example to our awareness of de novo mosaicism with a CCR and a normal cell line detected prenatally is that described in Hastings et al. (1999b), 46,XX,t(3;10)(p13;q21.1),inv(6)(p23q12)/ 46,XX, and this case was associated with fetal abnormality.

Inversion Mosaicism.

In four cases in Hsu et al. (1996) of diagnosis at amniocentesis of mosaicism for an inversion (pericentric or paracentric), 46,inv/46, the outcome was normal in all (all four were studied postnatally, with the mosaicism found in only one).

De Novo X or Y to Autosome Balanced Translocation

X-Autosome Translocation.

In the case of a de novo apparently balanced X-autosome translocation, there are the additional possible complications of *(a)* gonadal dysfunction if the breakpoint is within one of the critical regions of the X chromosome, and *(b)* the unpredictability of the patterns of inactivation with the possibility of severe abnormality. On theoretical grounds, the risk may be about twice that for the simple autosomal translocation given earlier (Waters et al., 2001), although Abrams and Cotter (2004), reviewing the literature, arrived at a risk figure as high as 50% (and disregarding a possible risk for reproductive health). Nevertheless, in the case they report, a normal daughter, with follow-up to age 17 months, was born after amniocentesis (for advanced maternal age) had shown a de novo 46,X,t (X;6)(q26;q23) karyotype, with the normal X late replicating. They, and we, hope that further such cases will be reported. Hatchwell et al. (1996) provide the particular example of a severe phenotype associated with a whole-arm X-autosome translocation.

On the specific issue of an Xp21 breakpoint, the question of Duchenne muscular dystrophy arises. Evans et al. (1993) actually showed normal dystrophin on a fetal muscle biopsy following detection at amniocentesis of an apparently balanced rcp(X;1) with the X breakpoint at p21, and so predicted the child would not have Duchenne/Becker muscular dystrophy; and their prediction proved to be correct. In a case of de novo 46,X,t(X;9)(p21.3;q22) diagnosed at amniocentesis, Feldman et al. (1999) showed apparent integrity of the dystrophin locus on FISH. Methylation analysis indicated preferential inactivation of the normal X. On these two observations, the couple decided to continue the pregnancy; but fetal demise occurred at 34 weeks, probably due to chorioamnionitis following premature rupture of membranes at 33 weeks. No fetal defects were seen; dystrophin staining of muscle was normal. Had microarray analysis been available, a clearer answer might have been forthcoming.

Yq-Autosome Translocation.

The balanced Yq-autosome reciprocal rearrangement, with a 46-chromosome count, has the gonosomal breakpoint in proximal Yq (the breakpoints usually given as q11, q11.2, or q12). Hsu (1994) reviewed 23 reports, in which the usual ascertainment was through infertility (oligospermia/azoöspermia) in the adult male, with a few being found incidentally and including one at prenatal diagnosis. Only three, including two from the early 1970s in which the detail of the rearrangement was less certain, were identified through a malformed child. It may be that such translocations should be regarded as conveying no greater risk for an abnormal intellectual phenotype than do reciprocal autosomal translocations, but acknowledging a frequent, perhaps inevitable compromise of fertility (p. 129).

In the particular case of a de novo translocation with Yqh material on the short arm of an acrocentric (which is, to be precise, an *un*balanced rearrangement), this is unlikely to be the basis of any phenotypic defect (p. 130).

De Novo Balanced Robertsonian Translocation

Heterologous Robertsonian Translocation.

The great majority of cases will be disomic nonmosaic and of biparental inheritance, and a normal phenotype is to be expected. The risk for phenotypic defect over and above the baseline is due to UPD and, theoretically, to occult mosaic trisomy.

Reviewing their own and others' data, accumulating some 102 prenatal cases, Ruggeri et al. (2004) determine a risk for UPD of about 3%, based upon the observation of three affected cases (all three due to upd14mat). This figure is a little higher than earlier estimates, in which results from inherited and de novo cases had been pooled (Silverstein et al., 2002). But because the UPD cases all fell within the de novo group, it may be prudent to regard these separately (and in that case, to see the risk for the inherited form as being very low). Shaffer (2006) combined all studies on heterologous Robertsonian translocations and found that, if all chromosomal combinations are considered, the risk for UPD was 0.8%. If only those imprinted chromosomes are considered (robs that include chromosomes 14 and 15), then the risk of UPD was 0.6%. De novo cases appear to have a two-fold increased risk (~2%) as compared to maternally inherited Robertsonians (~1%) or paternally inherited (no cases identified in the surveys). Although no cases of paternal UPD were identified in the prenatal surveys, there are single case examples of paternally derived robs and UPD. Given these data, it may be warranted to check for UPD, more especially in the setting of one of the imprintable chromosomes (14 or 15) being a component of the translocation. UPD 15 can be tested at prenatal diagnosis using DNA methylation analysis at the 5′ *SNRPN* locus (Glenn et al., 2000); amniocytes rather than chorionic villi may be the preferable tissue to test (Silverstein et al., 2002).

As for *occult mosaic trisomy*, this is a state that may have arisen from an initially trisomic 46,rob conception. The trisomic chromosome may then be discarded at a postzygotic "correction," with the conceptus now cytogenetically balanced (45,rob), but with the possibility remaining of mosaicism with an occult, or very low-level trisomic cell line(45,rob/46,rob). While this cannot absolutely be excluded, the recorded experience to date indicates that this is an exceptional complication, at least at a level that might have evident clinical consequences. No such case was discovered in a de novo rob in the series of Berend et al. (2000a) (there was a single case due to a *familial* translocation that did have low-level (4%) trisomy 13 mosaicism, along with UPD 13).

Homologous Robertsonian Translocation (or Acrocentric Long Arm Isochromosome).

A chromosome comprising two long arms of the same acrocentric chromosome may be either an homologous Robertsonian translocation, or an isochromosome: for example rob(13q13q)[8], or i(13q).

If the formation of an homologous rob has been through the fusion of the maternal and paternal homologs, which of course must have occurred as a postfertilization event, then the rearrangement manifestly has to be a true Robertsonian translocation, and the inheritance is *biparental*. In that case, a phenotypically normal child is the expectation, other things being equal (Abrams et al., 2001); infertility would, however, be anticipated (see Chapter 7).

All isochromosomes, and some homologous translocations, will display *uniparental* inheritance. The importance of uniparental disomy depends upon the chromosome involved. In Berend et al.'s (2000a) Robertsonian series, there were six identified with an homologous translocation, all de novo, and four of these had UPD, two upd(13)pat and two upd(14)pat. Barring isozygosity for a single gene mutation (see later), normal outcomes are to be expected following prenatal diagnosis of a Robertsonian translocation (isochromosome) comprising a chromosome not subject to imprinting (chromosomes 13, 21, 22). This is actually recorded for the i(13q) UPD (Berend et al., 1999). No prenatal diagnosis reports exist for i(21q) UPD or i(22q) UPD, but the postnatal state of normality in each of these is known (Engel and Antonarakis, 2002). Isodisomy for at least part of the chromosome will exist in the i(13q) UPD, i(21q) UPD, and i(22q) UPD states, and this raises the question of a risk, not readily quantifiable but likely very small, for a

mendelian autosomal recessive disorder due to isozygosity, the parent being heterozygous for the mutation in question. On the other hand, for the imprintable chromosomes 14 and 15, the risk for clinical defect is absolute following prenatal diagnosis of the rea(14) UPD and the rea(15) UPD, and the clinical syndromes of UPD14 or UPD15, maternal or paternal, would inevitably ensue (Berend et al., 2000a; McGowan et al., 2002).

De Novo Balanced Inversion (Pericentric or Paracentric)

(Note that a supposed "inversion" detected in this setting may actually be an unbalanced translocation). The risk from Warburton (1991) for phenotypic abnormality associated with a de novo inversion is 9.4%, which is 6%–7% over and above the background risk. The numerator is small, however, and the 95% confidence limits span 2%–25%. Since, in theory, a two-breakpoint inversion should not imply a greater risk than the two-break reciprocal translocation, the figure for this latter category as noted earlier, namely 3% (or a little above), might reasonably be seen as appropriate also for the inversion. Although if one breakpoint is in an acrocentric short arm, the risk might be that much less (Leach et al., 2005). In the special case of the X inversion, there may be gonadal insufficiency in the female (Dar et al., 1988; Dahoun et al., 1990). Pathology due to an inversion per se is rare, but well recognized; for example, Sotos syndrome consequential upon disruption of the *NSD1* gene, due to a de novo inv(5)(q33.3q35.3) (Malan et al., 2008).

De Novo Balanced Insertion

Only one case is recorded, to our knowledge, of a de novo apparently balanced autosomal interchromosomal insertion detected prenatally (Hashish et al., 1992). The child proved to be phenotypically normal. Van Hemel and Eussen (2000), in their review of nearly 90 families with an interchromosomal insertion, note that of the nine probands with congenital anomalies having a balanced insertion, seven were de novo and only two familial. It might reasonably be suggested that the risk for the interchromosomal insertion (three breakpoints) would be similar or possibly a little greater than the de novo apparently balanced reciprocal translocation (two breakpoints). Recalling the 3% risk figure associated with the latter, perhaps a percent point above this is a fair figure to offer for the risk of "unspecified malformation and/or intellectual deficit." Gonadal insufficiency may accompany the de novo intrachromosomal ins(X) (Grass et al., 1981).

De Novo Balanced Autosomal Ring Chromosome

The 46,r(A) ring chromosome is discussed in Chapter 11, and the reader is referred to specific instances listed therein. Rings that are truly balanced, reflecting a tip-to-tip telomere fusion, are nevertheless likely to cause growth retardation (or, in the case of r(20), epilepsy). Microarray analysis can reveal a very subtle deletion, which even targeted MLPA and FISH could not detect, as Manolakos et al. (2009) show in a case of ring 15 chromosome prenatal diagnosis (p. 205). Chen et al. (2006d) describe a specific example of a ring 9 detected at amniocentesis, with secondarily arising monosomic and trisomic cell lines flanking the disomic karyotype, 45,–9/46,r(9)/46,idic r(9), and in which subtelomeric deletions were demonstrated; post termination, fetal abnormality was evident. These authors report a similar story with a ring 4 (Chen et al., 2007a). Equally, array-CGH may demonstrate no apparent loss of material, as Papoulidis et al. (2010) report with a ring 21, the baby subsequently born being assessed as normal.

De Novo Unbalanced Structural Rearrangement

Unbalanced Rearrangement, Modal Number 46 or 45.

Autosomal.

For any de novo autosomal structural rearrangement in which cytogenetic imbalance can be demonstrated, serious phenotypic abnormality is highly likely. Often, it is not possible readily to identify the precise origin of a duplicated segment, which means that precise prediction of phenotype is not possible. Many cases, indeed most, are unlikely to be exactly the same as those in the literature or on the databases, and the counselor will need to make an informed evaluation. Ultrasonography may clarify the question if abnormalities are seen, but an apparently normal sonogram does not guarantee that the child would be normal (Al-Kouatly et al., 2002).

In the mosaic state, the risk may be high if pseudomosaicism is judged to be unlikely. Hsu et al. (1992) record 34 cases with at least one cell line having an unbalanced rearrangement (thus, presumed to be a true mosaicism). In follow-up studies, phenotypic abnormality was noted in about 50% and cytogenetic confirmation obtained in 65%. Each rearrangement needs to be considered on its merits. The dilemma of deciding how best to advise couples is illustrated in Cotter et al. (1998). They describe the karyotype 46,XX,der(4)t(4;5)(q34;q12)/46,XX detected at amniocentesis, imparting, in the abnormal cell line, trisomy for most of 5q. This was confirmed on two subsequent amniocenteses, with an average overall of 17% of amniocytes abnormal, but with a 46,XX result on fetal blood sampling, and normal ultrasonography. The parents were advised that "few data were available" to determine risk; they made a decision to continue the pregnancy. In the event, the child appeared normal at birth and at 2-year follow-up; 100 cells at cord blood karyotyping were normal. In contrast, 46,XX,add(15)(p10),t(2;15)(p10;q10)/ 46,XX mosaicism detected at 30-week prenatal diagnosis (performed due to IUGR), and shown on both amniocentesis and fetal blood sampling, was associated, post termination, with fetal anomalies consistent with a partial trisomy 2p (Pipiras et al., 2004). Cotter et al. rightly call for others' experience in similar cases to be published.

If a "*jumping translocation*" (p. 159) leads to imbalance, fetal defect is very probable (Annable et al., 2008).

X-Autosomal.

Prediction with respect to the unbalanced X-autosome translocation is precarious (and see Chapter 6). Albeit the pattern of inactivation may lessen the effect, and indeed convert an invariably lethal imbalance to a survivable state, the degree to which selective inactivation may occur in fetal tissues is not knowable, and a significant defect remains very probable (Kulharya et al., 1995; Garcia-Heras et al., 1997; Orellana et al., 2001). Had the child with an unbalanced der(X)t(Xp;22q) described on p. 127 (see Fig. 6–10 in Chapter 6) been identified at amniocentesis, and with the DiGeorge critical region intact and no inactivation on the 22q segment, a prediction of typical Turner syndrome might have been reasonable. In the event, this child proved to have a significant mental handicap. Contrary examples in which a prediction of major abnormality would have been mistaken are rare.

Y-Autosomal.

Autosomal material attached to the heterochromatin of a Y chromosome is to be seen in essentially the same light, as if it had been a translocation to an autosome (and see Chapter 6). A rare, but recurrent unbalanced karyotype seen at prenatal diagnosis is the t(Y;1)(q12;q21) translocation in mosaic state, which endows essentially a 1q trisomy in the tissue with the translocation (Scheuerle et al., 2005). The phenotype is lethal. Vice versa, if Y material is attached to an autosome, and if autosomal material is lost at that site, the autosomal monosomy of itself determines phenotypic defect (Klein et al., 2005).

A somewhat different, and very rare category, is that in which a near-intact Y, missing only part of the pseudoautosomal region, combines with an acrocentric chromosome. Borie et al. (2004) describe the prenatal diagnosis of 45,X,dic(Y;22)(p11.3;p11). Had this dicentric chromosome included all the Yp material, the child might have been normal. But in fact the *SHOX* locus, at Yp11.3 (see Fig. 6–1 in Chapter 6), was deleted, and the otherwise normal male child had short stature.

Yq;15p Variant.

In the population there is a common variant whereby the heterochromatin of Yq becomes translocated to the short arm of chromosome 15; this occurs in about 1 in 2000 individuals. Occasionally, other translocations will occur between these two chromosomes, such is the case reported by Chen et al. (2007), in which the father's karyotype was 46,X,t(Y;15)(q12;p13) and the female fetus inherited the abnormal chromosome 15. Because the derivative chromosome has deleted the repetitive 15 short arm and replaced it with Yq heterochromatin, no phenotypic effect would be expected. The authors suggest that methylation analysis for chromosome 15 should be considered, although in fact no cases of UPD 15 due to this common variant have been reported.

Unbalanced Rearrangement, Modal Number 47: Supernumerary Chromosome

A supernumerary chromosome may be of substantial size, and identifiable as to its makeup; or it may be smaller, and its origin uncertain. The latter are referred to as supernumerary marker chromosomes (SMCs), and these have also been described as marker, extra structurally abnormal chromosomes (ESACs), and accessory chromosomes (Hook and Cross, 1987a). The SMCs we mostly consider here are the small SMCs (sSMC); Liehr (2008) defines these as structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional banding cytogenetics alone, and which are generally equal in size or smaller than a chromosome 20 in the same metaphase spread. Some are quite harmless, and associated with phenotypic normality, and others are not: they are a very heterogeneous group.

SMCs are encountered in about 1 in 1000 prenatal diagnoses, frequently in the mosaic state with a normal cell line. Upon the discovery of an SMC at prenatal diagnosis, an urgent parental chromosome analysis is required. The majority will prove to be de novo. These questions are to be asked: From which chromosome is it derived, and does it comprise euchromatin or heterochromatin? Is it a recognized type of SMC, for which precedents are recorded? Precise characterization is necessary, and this requires the use of FISH or other stains (Liehr, 2008; Yaron et al., 2003), or of microarray analysis (Ballif et al., 2006; Pietrzak et al., 2007; Gruchy et al., 2008). On FISH, about 80% are shown to derive from one of the acrocentric chromosomes, most commonly chromosome 15 or chromosome 22, and often involving only the pericentromeric region and/or the satellites (Crolla et al., 1998; Lin et al., 2006).

De Novo Identifiable Supernumerary Chromosome of Substantial Size.

An additional chromosome which is of sufficient size that it can be characterized on initial routine analysis as a deleted or rearranged form of a specific autosome will imply a very high risk of abnormality, approaching 100%, due to partial trisomy of that chromosome. Once a supernumerary chromosome has been identified, it is no longer referred to as an SMC; it is now described as a ring or derivative, for example, r(7) or der(22)[9], or whatever may be the precise description.

Familial Supernumerary Marker Chromosome.

Interpretation in the case of a familial SMC is usually straightforward. If one parent is also 47,+SMC and phenotypically normal, it can be assumed no discernibly increased risk for fetal abnormality exists (Brøndum-Nielsen and Mikkelsen, 1995). Hastings et al. (1999a) surveyed a 10-year experience in London and report six familial SMCs, which included three that were 14 or 22-derived, an idic(15), a der(6), and a mosaic der (16). The outcomes in the five proceeding to live birth were all normal at follow-up from 5 months through 5 years. When the parent has the SMC in mosaic state, prediction for the fetus is more difficult: the chromosome could be potentially harmful, but the parent might have been protected by a particular tissue distribution. The mosaic der(16) in Hastings et al. comprised centromeric chromatin: mother and child, both mosaic, were normal. Each case will need to be judged on its merits. The idic(22) presents an exception, since a parent can be normal and the child abnormal (Crolla et al., 1997). A unique case of the parental SMC being seen in double dose at prenatal diagnosis is recorded in Wolstenholme et al. (1992). At CVS, amniocentesis, and in the normal child, the karyotype was 48,XX,+mar,+mar, from a 47,XX,+mar mother.

If the SMC is revealed as being a small derivative chromosome from 3:1 malsegregation, one parent being a balanced translocation carrier (Stamberg and Thomas, 1986), this is of course an entirely different story, and serious phenotypic abnormality is practically certain. Similarly, Nevado et al. (2009) warn of the need to beware of the sSMC from a carrier parent who has a cryptic deletion for the same material (see p. [link]). Baldwin et al. (2008b) document the prenatal diagnosis of a small ring 4, which was then shown to be carried (in mosaic state) in the mother, and the pregnancy continued. When the child was subsequently investigated for speech delay, in fact he was shown to be duplicated for the genomic material carried in the ring, he not having inherited his mother's “balancing” deleted chromosome 4.

De Novo Supernumerary Marker Chromosome.

De novo SMCs have been described for most chromosomes (Hastings et al., 1999a); two-thirds of small SMCs are acrocentric derived (Dalprà et al., 2005). The mode of ascertainment may suggest a category of risk: those in which fetal ultrasonographic anomaly has been detected would enter a higher risk group, as might, intuitively, those discovered through an increased-risk finding at maternal serum screening. The risk for abnormality is low in the very small derivatives of acrocentric chromosomes which stain negatively for euchromatin, and which may be satellited. If, however, a der(15) contains the segment of proximal 15q that includes the Prader-Willi/Angelman region, the risk is high (see later). Mosaicism appears not to alter the risk for abnormality.

With a reasonable level of cytogenetic characterization of small SMCs and ultrasound examination, it is possible to categorize most fetuses as being either at high risk of abnormality, or at a relatively low risk (less or much less than 5%). In principle, those comprising heterochromatin convey a low risk, while a euchromatic SMC may imply a high risk.

Published series of liveborn children with SMCs are mostly biased by ascertainment in favor of phenotypic abnormality. Series of prenatally diagnosed fetuses are deficient in that there is usually only a short-term follow-up of liveborn children, while pathological assessments following termination can only show major structural malformations (Warburton, 1991). Brøndum-Nielsen and Mikkelsen (1995) report a 10-year experience in Glostrup during which nine de novo SMCs were identified. In seven cases, termination of pregnancy was chosen, with some of these showing defects at pathological examination; and in the two pregnancies continuing, one infant with a minute acrocentric-derived SMC was normal at birth, while one with a ring-like 17 was “slightly retarded” at age 2 years. In the similar survey of Hastings et al. (1999a), data were presented on 31 prenatally diagnosed SMCs, of which 21 were de novo. In ten of these 21 proceeding to FISH analysis, six being mosaic, five were shown to be 15-derived and three 14 or 22-derived; the remaining two included a r(8) and a der(16). Of the six in which the pregnancies continued, only the r(8) child was physically and developmentally abnormal.

The minute marker (the *very* small SMC, minSMC) may comprise only centromeric material, and we discuss such a prenatal case on p. 421, a minSMC apparently comprising no more than chromosome 18 centromere; the child turned out to be normal. A similar example, although the sSMC not quite so tiny, and the child subsequently born being normal, is given in Sung et al. (2009); the sSMC comprised chromosome 10 material (and may actually have been a very small ring). Microarray in such cases might not be readily interpretable: that is to say, judgment about a possible pathogenicity may be uncertain. A 21-derived sSMC at amniocentesis, which might otherwise have been interpreted benignly, was seen in a different light due to an accompanying minor 47,XY,+21 cell line, leading to a conclusion that an initially trisomic 21 conception had generated a del(21q) cell line; upon posttermination, the fetal karyotype was 47,XY,+21/47,XY, +der(21)/46,XY (Stefanou and Crocker, 2004).

Specific Well-Characterized De Novo Supernumerary Chromosomes.

The Bisatellited Microchromosome.

These tiny chromosomes can be thought of as the reciprocal product of the Robertsonian rearrangement. They are typically harmless (Romain et al., 1981; Adhvaryu et al., 1998; Dalprà et al., 2005; Gruchy et al., 2008).

Isodicentric 15.

About half of all SMCs are an idic(15) (also referred to as pseudodicentric 15, or inverted duplication 15; and see p. [link]). These are typically dicentric and bisatellited, although one of the centromeres may be suppressed. The smallest ones (smaller than chromosome 21q) appear to be harmless, but larger ones result in the “idic(15) syndrome,” characterized by mental defect and autistic features. The boundary between smaller and larger is in 15q12. The use of D15S10 or *SNRPN* FISH probes, which recognize sequences in 15q12-q13, enables distinction of harmless and pathogenic chromosomes (Eggermann et al., 2002). We may anticipate an increasing role for microarray analysis (Wang et al., 2004). Rare idic(15)s have been associated with UPD 15, and it may be warranted to check for this possibility (Hastings et al., 1999a).

Isodicentric 22.

The bisatellited idic(22) typically, but not invariably, causes cat-eye syndrome. If the idic(22) lacks proximal 22q euchromatin, normality is very probable, whereas those containing euchromatin can lead to a phenotype anywhere between full cat-eye syndrome and normality (Crolla et al., 1997).

Autosomal Isochromosomes.

The mosaic state is usual for a supernumerary isochromosome, and thus the discovery of 47,+i/46,N is always a concern, whether at a level II or even level I mosaicism. Such a karyotype raises the prospect of an effective mosaic tetrasomy for the chromosomal arm concerned. A 46-chromosome karyotype in which one homolog is replaced by an isochromosome typically implies a trisomy for one arm of that chromosome, and monosomy for the other. These are certainly rare observations: in an amniocentesis-based survey from Italy, based on a little under 90,000 diagnoses, the most frequent were, in order, isochromosomes of 20q, 9p, 18p, and 12p, at approximately 1 in 30,000, 45,000, 45,000, and 90,000, respectively (Forabosco et al., 2009).

47,+i(5p).

Sijmons et al. (1993) assessed a dysmorphic and neurologically compromised child with a 5p isochromosome in 3/31 lymphocytes and 12/14 skin fibroblasts, and yet upon retrospective checking, only one of 217 cells from a stored short-term CVS culture was 47,XY,+i(5p). We contrast this unfortunate experience with ours of seven cases of i(5p) mosaicism identified at CVS, six of which went on to follow-up amniocentesis (Clement Wilson et al., 2002). Three children were followed up to 2½, 3¼, and 4 years, and their normality was quite apparent. In one of these children, a circumscribed area of the placenta following delivery karyotyped 47,+i(5p), adjacent parts karyotyped 47,+i(5p)/ 46,N, and most of the placenta (and the child himself) had a normal karyotype. The CVS sampling had presumably needled this small region of confined placental i(5p) mosaicism. One pregnancy tested 100% i(5p) at CVS, and the parents chose termination; no i(5p) cells were detected from fetal skin culture. In another with a 65% load at CVS, a follow-up amniocentesis showed 45% of cells with the isochromosome, and posttermination tissues showed 15%–30%. From the foregoing, we may conclude that a *CVS* diagnosis with a normal follow-up amniocentesis and with normal ultrasonography suggests, but cannot confirm, a normal child. As for the primary detection of i(5p) mosaicism at *amniocentesis*, only three cases are recorded, with all three having an abnormal outcome (Reddy and Huang, 2003).

47,+i(8p).

López-Pajares et al. (2003) review the small number of reported cases. Two examples are given of discordance between amniocentesis (normal) and postnatal blood (tetrasomy 8p), an unusual pattern for isochromosomes (but cf. the i(9p) later). A disconcerting story is told in Nucaro et al. (2006): i(8p) mosaicism was seen at long-term cultured (but not short-term) CVS, with a normal result after amniocentesis, but resulting in a child severely retarded and epileptic, and with a 5% level of the i(8p) on blood.

47,+i(9p).

The clinical picture, and the subtleties of different breakpoints, are reviewed in Dhandha et al. (2002). Isochromosome mosaicism can be the basis of a false-negative test result at prenatal diagnosis. Thus, Eggermann et al. (1998) reported an abnormal baby born to a 39-year-old mother, in whom amniocentesis at 14 weeks gestation had returned a normal karyotype. On blood analysis, the child had an i(9p) in 32% of cells. From one skin biopsy, 50 cells had a normal karyotype, but on a second biopsy, 5 out of 8 cells showed the i(9p) chromosome. The particular attribute of the i(9p) is for blood, but not skin, to show the abnormality, and this may provide the explanation for its nondetection at amniocentesis. Pertile et al. (1996) support this interpretation, in their follow-up of a (nonmosaic) CVS diagnosis of idic(9)(q13). An extensive search at amniocentesis revealed a single abnormal colony, which might well otherwise have been missed. Finally, fetal blood sampling showed the idic(9) in 8% of cells. A more severe case is recorded in Tang et al. (2004), which showed the isochromosome in all amniocytes at 24 weeks and most blood and fibroblast cells from the multiply malformed infant (who died at 1 month of age).

47,+i(10p).

A single case is on record, the diagnosis having been made following the recognition of fetal defects on ultrasonography (Wu et al., 2003).

46,i(13q).

A de novo “Robertsonian” translocation, leading to trisomy 13, is, in the majority of cases, actually an isochromosome (Bugge et al., 2005).

47,+i(12p).

The 12p isochromosome is the basis of the Pallister-Killian syndrome. The fractions of abnormal cells detected at prenatal diagnosis can vary greatly. Bernert et al. (1992) showed in one example 100% of short-term CVS cells and 10% of amniotic fluid cells having the 47,+i(12p) karyotype, whereas in Kunz et al. (2009), at CVS the isochromosome was seen only in long-term culture; in both cases, the pregnancies were terminated. Horn et al. (1995) reported a pregnancy in which CVS gave a 46,XY result on direct (17 cells) and cultured (8 cells) analysis (and 28 further cells on a retrospective study), and the abnormal newborn baby was 46,XY on a peripheral blood study (100 cells counted); at 18 months, a clinical diagnosis of Pallister-Killian syndrome was made, and the karyotype on skin fibroblast culture was 47,XY,+i(12p)/ 46,XY, with 85% of cells having the isochromosome. (Had it been an amniocentesis rather than CVS that had been done, abnormal cells would probably have been seen.) While classical karyotyping typically returns a normal result, array-CGH is able to detect subtle mosaicism in this tissue (Theisen et al., 2009).

47,+i(18p).

Schinzel (2001) notes that over 75 cases of 47,+i(18p) have been recorded. Multiple physical anomalies and a moderate to severe degree of mental retardation characterize the clinical picture. Boyle et al. (2001) emphasize the plausibility of a premeiotic origin, and the caution therefore that gonadal mosaicism may exist in a parent, as they illustrate in their report of affected half-sisters.

46,i(18q).

The karyotype produces a combination of monosomy 18p and trisomy 18q. Chen et al. (1998) record that many 18q isochromosomes diagnosed prenatally are associated with very severe malformation, such as holoprosencephaly and cloacal dysgenesis. Levy-Mozziconacci et al. (1996) describe a case presenting at 22 weeks gestation with abnormal ultrasonography, and although the direct CVS was 46,XX in all cells, amniocentesis and fetal blood sampling showed the isochromosome (an isodicentric, in this instance) in all cells: an example of complete CVS-amniocentesis discordance.

46,i(20q).

An i(20q) identified at amniocentesis in mosaic form appears most often to be a benign finding, a rather surprising conclusion. It may be an unusual sort of mosaicism in being confined, or largely so, to amniocytes, the abnormal cell line having arisen as a postzygotic event, and its growth perhaps favored in vitro (Robinson et al., 2007). The few reported cases with fetal defect could reflect a tissue distribution which included the fetal anatomy. Goumy et al. (2005) counsel caution, and point to the advisability of careful ultrasonography, targeted in particular to the brain and vertebrae.

46,i(21q).

This rearrangement is an isochromosome, not a Robertsonian translocation (Shaffer et al., 1991). The phenotype is that of Down syndrome. Gilardi et al. (2002) report a case in which the isochromosome probably arose postzygotically in an early cell destined to form the lineage of the inner cell mass and the extra-embryonic mesoderm, such that a

direct CVS gave a nonmosaic 46,XX result, while long-term CVS and posttermination fetal studies showed nonmosaic 46,XX,i(21q), and a similar story comes from Brisset et al. (2003). The i(21q) can also exist in a 47-chromosome karyotype. Nagarsheth and Mootabar (1997) showed a 47,XY,+i(21q)[6]/46,XY[19] karyotype at amniocentesis; the parents elected to continue the pregnancy, and the abnormal child had only one out of 120 peripheral blood lymphocytes with the i(21q), the other 119 being normal. These authors suggest that some previously reported cases of supposed i(12p) mosaicism may have been, in fact, i(21q).

47,+i(22q).

A single case of an isochromosome for 22q being detected at amniocentesis is recorded in Guzé et al. (2004). The isochromosome was probably generated postzygotically, with the subsequent production of additional abnormal cell lines. The pregnancy continued to full term: the child had several defects and died on the second day of life.

De Novo Supernumerary Ring Chromosomes (Autosomal).

Autosomal ring chromosomes imply a high risk of phenotypic abnormality. They originate from a variety of chromosomes and contain euchromatin. Certain of these, in which only one arm of the chromosome is represented in the ring, are specifically recorded in association with phenotypic abnormality: r(1p), r(5p), r(7q), r(8q), r(9p), r(10p), r(20p), r(20q) (Anderlid et al., 2001). The r(8) with an abnormal outcome in Hastings et al. (1999a) is mentioned earlier. Uniparental disomy may complicate the picture: James et al. (1995) and Anderlid et al. (2001) report supernumerary rings, from chromosomes 6 and 9, associated with UPD 6 and UPD 9, respectively. Very small rings, that might also be categorized as “small supernumerary marker chromosomes” (sSMCs; see earlier) might not necessarily cause an abnormal phenotype: for example, two infants in Kitsiou-Tzeli et al. (2009) born following prenatal diagnosis of 46,N/47,+r(20) mosaicism were judged normal in early infancy.

Chromosomal Breakage Resembling the ICF Syndrome.

The chromosomes at CVS may on occasion somewhat resemble the “starburst” appearance in the ICF syndrome (p. 347, Fig. 21–4). Ehrlich et al. (2001) noted that “anecdotal observations of these types of pericentromeric chromosome 1 and 16 anomalies in normal CVS metaphases are common” and concluded that “ICF-like chromosomal abnormalities are part of the normal spectrum for CVS chromosomes and need not indicate any clinical condition,” a conclusion supported by subsequent study (Tsien et al., 2002).

Presumed Normal Variants.

Chen et al. (2006c) review the question of variants detected at prenatal diagnosis. They identified 16 variants of euchromatin or heterochromatin in 21,832 amniocenteses. Eight of nine euchromatic variants were proven inherited, and seven were C-band positive. The remaining C-band positive, heterochromatic variants were all inherited from a carrier parent. Concerning the specific case of the NOR translocation, or interstitially inserted satellite, and as noted in Chapter 16, “genetic counseling should be reassuring” if this is discovered at prenatal diagnosis (Faivre et al., 1999, 2000a; Chen et al., 2004b). The Y;15 variant is noted earlier.

Sex Chromosome Abnormalities

Full Aneuploidy

A sex chromosome abnormality is not an uncommon discovery at prenatal diagnosis, with an overall incidence of 1 in 250–300 (Linden et al., 2002). The main conditions are XXY, XXX, XYY, and 45,X. As Boyd et al. (2011) write: “The importance of providing parents with accurate information about the frequency of the diagnosis and the variability of the condition on the basis of outcomes from unbiased population-based follow-up studies on the specific chromosome abnormality cannot be overemphasized.” Two of these aneuploidies (XXY and 45,X) may be firmly predicted in terms of an abnormality of development of the reproductive system. Children with Klinefelter and 45,X Turner syndrome will with near-certainty be infertile. For those couples deciding to continue a pregnancy, Robinson et al. (1986) offer a useful commentary. Parents of children predicted to be infertile might feel a sense of loss—a “sadness and regret about their child's anticipated loss and about their own loss of grandchildren” and “concern about their children's wholeness and, by extension, their own.” Parents may take some comfort from knowing that infertility is by no means an uncommon problem in the general population, and further comfort from the advice that recent advances in artificial reproductive technology may now enable the infertility to be overcome, in some individuals.

The picture for intellectual and psychological functioning is less predictable. Earlier adult studies defining a strong association with mental deficiency and psychological disturbance were contaminated by ascertainment bias (and counselors' personal experience may have been more with those children whose problems were sufficiently severe that they had come to medical attention). Children identified in newborn populations screened for cytogenetic abnormalities and subsequently followed up constitute a group unbiased in their ascertainment, although perhaps subject to other but less important biases (Puck, 1981). Data from the study of such children in several American and European cities, followed from infancy through childhood, adolescence, and young adulthood, have now given a reasonably clear picture of the natural history of the more common sex chromosome aneuploidies (Linden et al., 2002). In general, the IQ averages 10–15 points below that of the siblings. Hook's (1979) early proposition has held up: some sex chromosome aneuploidies influence brain function in such a way that the development of intellectual capacity, emotional maturity, and speech and language skills are affected to some extent; but none of these effects necessarily occurs, none is specific to sex chromosome aneuploidy, and some may be amenable to corrective intervention. There is considerable overlap with the XX and XY population.

Ratcliffe (1999) and Bender et al. (2001) provide long-term follow up data, well into adulthood. Bender et al. followed eight 45,X, ten 47,XXX, and eleven 47,XXY individuals through to an age range of 26–36 years, using siblings as controls, and noted the IQs of the aneuploid groups to be considerably less compared with the sibs. Nevertheless, the variation is wide, and these authors emphasize the point that “sex chromosome aneuploidy does not exert its influence in a vacuum, but rather interacts with the host of other genetic and environmental influences that collectively guide human development.” Children with sex chromosome aneuploidies seem more susceptible to either the good or the bad effects of a stable or of a dysfunctional family setting than do their 46,XX and 46,XY siblings (Stewart et al., 1990; Bender et al., 1995). Children identified at prenatal diagnosis, a group biased toward higher socioeconomic status, may do better academically and socially than the cohorts followed from birth, although it was nevertheless true in the study of Linden and Bender (2002) that these children had “a strong risk for developmental problems, particularly for learning disabilities … [albeit that] these problems were not often severe.” There may, however, be an increased risk for psychosis in childhood and adulthood (Kumra et al., 1998).

If a couple decides to continue the pregnancy, what should they say to others? Should the family know, should they tell friends, and should school personnel be aware? And when should the child learn about his or her chromosomal condition? Linden et al. (2002) have considered these questions, and in general make a case for openness within the family, but see no need, indeed potential disadvantage, for those outside to be told.

We next outline the predicted outlook for the more commonly encountered sex chromosome aneuploidies. Attention is paid mostly to gonadal function and to intellectual and social development.

XXY (Klinefelter Syndrome)

Almost certainly, the child becomes an infertile adult, although in recent times testicular sperm extraction with in vitro fertilization (IVF) has enabled a small number of men to become fathers (p. 224). Penile size is usually normal; the testes will be small. Androgen deficiency can be managed by replacement therapy with testosterone. It may be that treatment induces a more masculine body habitus, improved self-esteem, vitality, ability to concentrate, and sexual interest (Nielsen, 1990; Winter, 1990). Gynecomastia may be present, transiently, in some 50%; if it persists, it can be treated surgically.

Verbal IQ is reduced by some 18 points, and performance IQ by 11 points (Leggett et al., 2010). Learning difficulty at school is to be expected. Of 13 XXY boys studied by Walzer et al. (1990), 11 had persistent reading and spelling problems. Bender et al. (1993) note that a deficit in verbal fluency and reading is “the most homogeneous and consistent cognitive impairment found in any sex chromosome abnormality group,” and this may reflect a specific dysfunction of the left cerebral hemisphere. Specific

characteristics included a lowered level of motor activity, a pliant disposition, and a cautious approach to new situations; thus, in the classroom setting, they are perceived as "low-key children, well liked by their teachers, and presenting few behavioral management problems." Leggett et al. (2010) conclude that these boys "do not usually have major problems with social interaction and adaptation, although they may be timid and unassertive." Speculatively, the neural substrate of this passivity may reside in an underdevelopment of the amygdala, a brain nucleus that underpins aspects of social processing (Patwardhan et al., 2002).

Six Danish XXY boys were followed from birth to age 15–19 by Nielsen and Wohlert (1991), and all but one needed remedial teaching. Their career plans were carpenter, draughtsman, gardener, unskilled laborer, mechanic, and undecided. Stewart et al. (1990) comment that "XXY boys are unlikely to reach a level of personal and social development that is consistent with their family background." Ratcliffe (1999) commented upon a rate of psychiatric referral being above that of male controls (26% cf. 9%), with the neurotic score (not the antisocial score) being higher. (She also notes anecdotal mention of men from a Klinefelter clinic with professions including physician, engineer, minister, and accountant.) In a summary of psychosocial adaptation from several studies, recurring adjectives to describe the XXY personality were shy, immature, restrained, reserved. In the Denver study, 11 young adults with XXY "appeared to have met the demands of early adulthood with fair success, although slightly less well than did their siblings"; they appeared to have a diminished insight into their own psychology (Bender et al., 1999). Their mean IQ of 91 compared with 109 in normal male sibling controls. We have noted earlier the ameliorative effect of growing up in a stable and supportive family.[10]

XXX

A full literature review of the XXX syndrome is provided by Otter et al. (2010), and the reader will find this helpful. Physical development of the XXX female is generally unremarkable, although there is a tendency toward tallness. Gross and fine motor skills are likely to be somewhat impaired, and children are awkward and poorly coordinated. Pubertal development and fertility appear, for the most part, uncompromised. In a very few, genitourinary malformations (ovarian, uterine, renal, bladder) are recorded, of which the karyotype may or may not have been causal (Haverty et al., 2004; Linden and Bender 2004). It is the neural substrate in which the important vulnerability applies (and which may reflect a reduced rate of cell cycles during neurogenesis; Otter et al., 2010). Thus, major concerns in childhood relate to intelligence and language development and poor self-confidence, and, in adulthood, psychosocial maladjustment and, occasionally, frank psychiatric disease. Full scale and verbal IQ is reduced by some 10–20 points. Language comprehension and use of speech are impaired in over half the cases. Learning difficulty is likely and many will benefit from additional remedial teaching, but few require education outside the mainstream. In one small study of 11 girls, 9 needed special education intervention, and one was placed in a class for retarded children (Bender et al., 1993).

Harmon et al. (1998) and Bender et al. (1999) reported a longer follow-up in these young women, into adolescence and young adulthood, and documented difficult adaptation to the stresses of life. On a measure of social adjustment (in work, leisure, family, marital, parental), the XXX women scored significantly less well than their sisters. Their mean IQ was 82 (cf. sisters, 103). However, Ratcliffe (1999) described most XXX young women in the Edinburgh survey as "physically attractive, and displaying a common sense attitude that counterbalanced their low educational achievements" (and relieved to be free of the pressure they had felt while at school). The observations in the similar study of Rovet et al. (1995) were more promising, although, as Harmon et al. point out, this was a group from a higher socioeconomic stratum, and presumably both genetic and environmental factors would have been more favorable. An XXX girl who might otherwise have had an IQ of 130 can yet do well in spite of a reduction to 110; to the contrary, a drop from 90 to 70 would be a considerable handicap. Many counselors will know from their own experience how variable can be the phenotype.

XYY

The multicenter prospective study documented in Evans et al. (1990) reviewed progress in 39 boys and young men. The particular physical attribute of the XYY male is increased stature. Sexual activity is normal, and fertility is apparently uncompromised. Motor proficiency may be impaired. While the IQ is in the normal range, it is usually lower than those of sibs or controls, and about half of XYY boys have a mild learning difficulty, and may display poor attentiveness and impulsivity in the classroom. It may be that the aneuploidy causes a minor and subtle impairment of neurologic maturation, leading to some features of minimal brain dysfunction (Theilgaard, 1986). The vignettes from the series of Ratcliffe et al. (1990) of 10 Scottish subjects who had left school give an idea of what XYY young men are capable of: one ran a market stall, two are chefs, and the others were a private in the army, a waiter, a supermarket assistant, a video shop assistant, a technician, a laborer, and one training as a painter and decorator. In a cohort of children aged 8–16 selected for the XYY karyotype having been diagnosed prenatally, and of higher socioeconomic status, a considerable range in academic ability was observed, with most coping satisfactorily, and IQs ranging from 100 to 147 (Linden and Bender, 2002).

Perhaps the major concern is in psychosocial adaptation. These boys can have a low frustration tolerance, and some are prone to temper tantrums in childhood progressing to aggressive behavior in teenage and may need help to learn to cope with this. They may find it difficult to "read" social situations, and antisocial behavior is more common (Ratcliffe, 1999). The functioning of the family may be as much an ingredient as the karyotype in psychosocial development. Fryns et al. (1995) identified 50 XYY males among 98,725 patients referred for chromosomal analysis, and they note that this fraction of 50/98,725, approximately 1/2000, is very close to the newborn incidence, and thus drew a conclusion that the XYY phenotype differs little from the norm. They do, however, acknowledge a high (86%) risk for psychosocial pathology in those XYY males with concomitant borderline intelligence or frank mental deficiency. In Ratcliffe's follow-up report into adulthood (1999), some disconcerting data are noted, not incongruent with the conclusions of Fryns et al. Psychiatric referrals were five-fold compared with male controls (47% cf. 9%), and the rate of criminal conviction four-fold, the mean IQ of those convicted being lower than those who were not (although most offences were minor, and against property rather than persons).

45,X (Turner Syndrome)

Unlike the foregoing aneuploidies, monosomy X has a very high in utero lethality, peaking at around 12 to 15 weeks gestation. Spontaneous abortion follows amniocentesis-detected 45,X in three-quarters of cases (Hook, 1983). But some survive pregnancy and are born as infants with Turner syndrome. Robinson et al. (1990) note that "variability among 45,X girls is considerable; and precise predictions about any child's prognosis are not possible." They also emphasized that "a supportive environment that provides stimulation and encouragement is of considerable importance." These traits comprise the core phenotype (and a full description is given in Sybert, 2005):

- Gonadal failure with infertility is almost certain (Lippe, 1991). In the survey of Sutton et al. (2005), infertility was seen, by the women with Turner syndrome themselves, as the most concerning component of the phenotype. Classically, a spontaneous onset of puberty, with breast development and onset of menses, has been regarded as being very infrequent, although Pasquino et al. (1997) proposed that the fraction who enter a spontaneous puberty may be as high as 9%, and they suggest that earlier figures may have been biased downward by a policy, previously, of not karyotyping short girls who had had an onset of menstruation. Childbearing via ovum donation may be successful in some cases (Chapter 13). Pavlidis et al. (1995) reviewed sexual functioning in women with Turner syndrome and suggest strategies to avoid possible difficulties.
- Stature will be short. In a study of adult Danish women with Turner syndrome, never having had growth hormone therapy, the average height (with standard deviation) was 147 cm ± 7 cm (4 feet 10 inches ± 2½ inches) (Gravholt and Naeraa, 1997), which may be a little taller than in some other populations. A useful increment can be achieved with growth hormone treatment.
- Neuropsychological functioning is impaired. The IQ is reduced compared to siblings. At long-term follow-up in the Denver cohort (Bender et al., 1999), nine young women with 45,X had a mean lower IQ (85) compared with normal female sibling controls (104). Their educational achievements were, however, better than those of the XXX women from the same study: eight were high school graduates, and five had college degrees. In one notable case, Reiss et al. (1993) report monozygous twins, one nonmosaic 45,X and the other 46,XX, the former's performance IQ being 18 points less than her sister, but the verbal IQs practically the same. Psychological assessment indicates a particular vulnerability in social adaptation (Bender et al., 1999). Reiss et al. (1993) review aspects of the cognitive-behavioral phenotype and correlate the specific feature of difficulty with visual-spatial appreciation with a lesser volume of the right parietal cerebral cortex. Romans et al. (1998) confirmed and extended this appraisal in a study of 99 subjects with Turner syndrome, in whom they identified diminished abilities on measures of spatial and perceptual skills, visual-motor integration, recognition of facial expressions associated with a particular affect, visual memory, attention, and executive function (the ability to plan, organize, monitor, and

execute multistep problem-solving processes); the amygdala (see also XXY, earlier) may be the vulnerable neural substrate in this respect (Burnett et al., 2010). These traits are not improved by taking estrogen (Ross et al., 2002).

- Certain physical defects are associated, of which the major are neck webbing and coarctation (narrowing) of the aorta.
- Morbidity in adult life is increased (Gravholt, 2001; Swerdlow et al., 2001). Certain common diseases are more frequently seen: obesity, both insulin-dependant and insulin-resistant diabetes, hypothyroidism, heart disease, hypertension, stroke, and liver cirrhosis. Weakness of the bones (osteoporosis) implies a risk for fracture. There may be a place for ongoing hormone replacement therapy.

There is a possibility that Y-chromosome material may be present even if the karyotype is apparently nonmosaic 45,X. Huang et al. (2002) reviewed 74 cases of 45,X diagnosed prenatally, most having been ascertained via, or discovered with, abnormal fetal ultrasonography. Of six with normal ultrasonography, three showed a male genital phenotype. The explanations, upon more detailed analysis, were as follows: in one, a segment of Yp was translocated to a chromosome 14, shown on FISH with an SRY probe, and in the other two, there was low-level mosaicism for an idic(Y) marker. Normal male children were born. Some women with Turner syndrome who are 45,X on karyotyping may actually show Y sequences on molecular study, and these women do have a greater risk for gonadoblastoma (Mendes et al., 1999).

Sex Chromosome Polysomy

This category is to be seen as quite separate from 47,XXX and 47,XXY. Linden et al. (1995) review the phenotypes of 48,XXXX, 48,XXXY, 48,XXYY, 48,XYYY, 49,XXXXX, 49,XXXXY, 49,XXXYY, 49,XXYYY, and 49,XYYYY, and 48,XXYY, 48,XXXY, and 49,XXXXY are also outlined in Visootsak and Graham (2006). In each, variable intellectual compromise is characteristic. While the authors’ comment is well taken that the current perception of the seriousness of phenotypic abnormality may have been overstated due to ascertainment bias, and indeed they describe normal (but low) IQs in some of the 2n = 48 karyotypes, it remains true that most have substantial handicap due to intellectual deficit and abnormal behavior (Cammarata et al., 1999). The very rare 49,XYYYY karyotype is described in Paoloni-Giacobino and Lespinasse (2007); almost as rare is 49,XXXXY, concerning which Peitsidis et al. (2009) review prenatal diagnosis, noting that nuchal thickening is a frequent observation. IQ in both these pentasomies is very low.

XX Male.

Discordance of 46,XX karyotype and male genital phenotype, as seen on ultrasonography, allows prenatal diagnosis of the XX male (Trujillo-Tiebas et al., 2006). Other possible diagnoses need consideration, including congenital adrenal hyperplasia with virilization of a female fetus (Lonardo et al., 2009); of course the possibility of maternal cell contamination will need to be excluded. Normal intelligence and stature are predicted, but there will be testicular deficiency with infertility (Margarit et al., 1998).

X and Y Chromosome Mosaicism

True mosaicism involving the sex chromosomes seen at prenatal diagnosis presents a challenge in interpretation, and skilled ultrasonography, with respect to external genital anatomy, is central in determining the fetal gender. The problem is that the tissue analyzed at prenatal diagnosis may or may not reflect the distribution in the gonad. The presence of a Y chromosome in at least some gonadal tissue—or to be precise, the presence of the Y-borne *SRY* gene—will promote testicular development, which might or might not be complete, and which might or might not secrete male-inducing hormones. Thus, we may observe gender states from normal (although possibly infertile) female, through Turner-like female, genital ambiguity, mixed gonadal dysgenesis, even ovotesticular disorder of sex development (p. 336), to male with incomplete pubertal development, and to normal (although often infertile) male.

XX/XY.

This is usually pseudomosaicism, resulting from the growth of maternal cells in a 46,XY pregnancy (Worton and Stern, 1984). (Obviously, such pseudomosaicism would normally be undetected if the fetus is female.) Level III XX/XY mosaicism, curiously enough, is most likely to indicate a phenotypically normal female fetus in which the XY source is unknown, particularly when the XX cells predominate. A male “vanished twin” is a theoretical possibility (Worton and Stern, 1984), and indeed a quite plausible explanation, given the frequency with which a twin pregnancy prior to 7 weeks is followed, some months later, by the birth of a singleton baby (Sampson and de Crespigny, 1992). Analysis of placental membranes after delivery in a pregnancy from which one twin has “vanished” can often reveal a fetus papyraceous or a remnant empty sac (Nerlich et al., 1992). One can imagine tissue of the (male) twin remnant having been, by chance, in the path of the amniocentesis needle that sampled cells from the remaining (female) fetus. A girl born following such a prenatal diagnosis (Hunter et al., 1982) was followed through to mid-adolescence, and her development was entirely normal (A. G. W. Hunter, personal communication, 2002). A similar case was studied by I. Hayes and A. George (personal communication, 2009), with an XX:XY ratio of 90:10 on FISH of uncultured amniotic fluid, although nonmosaic 46,XX on cultured cells; ultrasonography indicated female external genital morphology. Following the birth of a normal girl, examination of seven sites from the placenta, and one site each from the cord and sac, all revealed a nonmosaic female sex complement, as did the cord blood sample.

A true fetal XX/XY karyotype is rare indeed, and it is more likely due to the fusion of two gametes; that is, chimerism (but other mechanisms exist; see Chapter 20, section on “Ovotesticular Disorder of Sex Development”). Presumably depending upon the gonadal distribution of XX and XY cells, the genital anatomy will be male, female, or in between. Malan et al. (2007) report XX/XY chimerism at prenatal diagnosis, the child (subjected to pelvic ultrasonography) proving to be an apparently normal girl. Ovotesticular DSD, with imperfect or ambiguous genital anatomy, has been recorded from an XX/XY amniocentesis result, with the same karyotype demonstrated in the child (Amor et al., 1999; Chen et al., 2005c, 2006f; Malan et al., 2007). Yaron et al., (1999) had a case presenting at amniocentesis, with normal male morphology on ultrasound. The XX/XY mosaicism was confirmed on a second amniocentesis, and, in due course, on the normal male newborn infant (including on genital skin). Amor et al. note the point that intellectual compromise is not to be anticipated. Hughes et al. (2006) provide guidelines on management for children with intersex conditions. Infertility is predicted; but remarkably enough, one XX/XY man has fathered a child, following IVF with retrieved sperm (Sugawara et al., 2005).

X/XY.

Patients coming to medical attention and found to have 45,X/46,XY mosaicism range in phenotype from females with classical Turner syndrome, through infants with ambiguous genitalia, to normal but infertile males (Telvi et al., 1999; Tho et al., 2007). A risk for gonadal tumor applies (Müller and Skakkebæk, 1990; Müller et al., 1999). By contrast, a normal male infant is the outcome in the considerable majority (90%–95%) of X/XY gestations detected at prenatal diagnosis—in other words, cases whose ascertainment was unbiased—and going through to birth (Hsu, 1994; Huang et al., 2002). Fertility, however, is likely to be compromised. Van den Berg et al. (2000) report a case in which nonmosaic 45,X was diagnosed at short-term CVS, with a nonmosaic 46,XY karyotype seen on long-term culture. Subsequent amniocentesis revealed a true 45,X/ 46,XY mosaicism. Post termination, fetal testing showed X/XY mosaicism in all tissues sampled (including gonads). Of 14 pathology studies on fetuses post termination in Chang et al. (1990), two were found to have ovotestes and one had a “precancerous” lesion.

Other Sex Chromosome Mosaicism.

The karyotypes most frequently seen are 45,X/ 46,XX, 47,XXY/46,XY, 45,X/47,XXX, and 47,XXX/ 46,XX. It appears that the considerable majority of cases of true sex chromosomal mosaicism of these types are associated with concordant (Y→male, no Y→female) and normal genital development (Hsu and Perlis, 1984; Wheeler et al., 1988). In X/XX, X/XXX, XXX/X/XX, and XXX/XX mosaicism IQ is not discernibly affected; verbal IQ may be slightly lowered in XXY/XY (Netley, 1986; Bender et al., 1993). Huang et al. (2002) reported a case each of X/XXX and XXX/X/XX mosaicism diagnosed at amniocentesis, the former pregnancy producing a newborn with features of Turner syndrome, and the other a normal female.

X/XX.

Koeberl et al. (1995) record 12 cases of 45,X/46,XX mosaicism detected at amniocentesis, with the percentage of 45,X cells in 10 of these being in the region of 20%–70%. Postnatal studies (blood and/or skin) confirmed the mosaicism in nine (at a lesser percentage in all but one), while in three of the children no 45,X cells were seen. None showed growth retardation postnatally and in none would a clinical suspicion of Turner syndrome have arisen. Two cases of presumed early ovarian dysfunction, one of these also having urogenital anomalies, might reflect an effect of the karyotype; it is possible some of the remaining cases could also manifest abnormal ovarian function at a later age. The abnormal neurology in one of the twelve is of uncertain significance. Koeberl et al. comment that "the prenatal diagnosis of 45,X/46,XX is not necessarily benign." Hsu (1996) and Sybert et al. (1996) debate the validity of previous larger series of X/XX prenatal diagnoses. A clear point forthcoming is that data from longer term follow-up are desirable, given that a functional gonadal component of the syndrome might not manifest until well into adolescence or adult life.

In 2002, Huang et al. reported their experience with 17 cases of X/XX mosaicism at amniocentesis. The ratios of X to XX cells ranged from 2:23 to 12:3. One case with intrauterine growth retardation (ratio 6:12) terminated in stillbirth, while the remaining 16 had normal ultrasonography. Of the eight cases continuing to term and for which information was available, two liveborn babies had the features of Turner syndrome (ratios 7:10 and 3:14), with the mosaicism confirmed postnatally in one of these. The remaining six (ratios ranging from 3:15 to 12:8) "reportedly had a normal female phenotype." To quote Huang et al. "the percentage of 45,X cells in amniocytes does not seem to be an indicator of pregnancy outcome as there was considerable overlap between cases with normal and abnormal outcome." In a unique case of a monozygous twin pregnancy, one fetus showed nuchal swelling and the other appeared normal (Gilbert et al., 2002). Fetal blood sampling showed low-grade 45,X[2]/ 46,XX[23] mosaicism in the former, and a normal 46,XX karyotype in the latter, in contrast to postnatal skin fibroblast karyotyping results of nonmosaic 45,X and 45,X[2]/46,XX[78], respectively.

X/XXX/XX and X/XXX.

One reported case of X/XXX/XX mosaicism illustrates the difficulty in extrapolating the distribution of cell types from one tissue to another (Schwartz and Raffel, 1992). Amniocentesis gave the proportions 16:20:64, respectively. Cord blood gave similar findings, although in placental tissue (chorion) the percentages were 2:41:57. The baby appeared normal. Sybert (2002) reviewed hers and others' data, and concluded that about 60% of girls with X/XXX/XX and X/XXX could be predicted to have short stature, and that "it is fair to suggest that residual ovarian function is possible and to caution that premature ovarian failure is common."

X/XYY and X/XYY/XY.

The X/XYY and X/XYY/XY mosaic states are (necessarily) abnormal in postnatally ascertained cases, but prenatally diagnosed cases have consistently manifested an apparently normal male genital phenotype, albeit that the mosaicism may be confirmed in the child subsequently born (Pettenati et al., 1991; Hsu, 1994). Presumably according to the distribution of X and XYY tissues, the gender in X/XYY mosaicism can be of either sex, or there can be ambiguity, these three states documented in one of the earliest reviews (Mulcahy et al., 1977). There is a tumor risk, and gonadoblastoma was identified at gonadectomy in a virilized female with mixed gonadal dysgenesis (Gibbons et al., 1999). Infertility is likely, but may be treatable (Dale et al., 2002). It is hypothetical whether the XYY line, if involving the brain, would determine an intellect and psyche of the XYY "syndrome" (earlier).

Structurally Abnormal Sex Chromosome

X-Y Translocation.

The most common form of the t(X;Y) has the X breakpoint at or distal to Xp22, and the Y breakpoint at Yq11.2. The intact sex chromosome may be an X or a Y chromosome, and the two states differ as follows.

46,X,der(X)t(X;Y): A de novo X-Y translocation would be expected to herald a female child, who will likely be short, 150 cm or less in height as an adult (Joseph et al., 1996; Speevak et al., 2001). The site of the breakpoint can be pinpointed with probes for two loci (steroid sulfatase, Kallmann syndrome) in Xp22.3; if these loci are present on the der(X)t(X;Y), intelligence and fertility may be intact, and other defects are unlikely. A few de novo cases have been associated with major defects, presumably due to a marginally more proximal Xp breakpoint, with the deletion of crucial genes.

46,Y,der(X)t(X;Y): If the intact sex chromosome is the Y, the child is expected to be male. If the probes noted earlier are present, the phenotype is likely to be confined to short stature and infertility. A more extensive loss of loci might determine a nullisomy that would cause important malformation and be lethal in utero. (Unbalanced X-autosome and Y-autosome translocations are dealt with in the section earlier on autosomes.)

Other rare types include dicentric X;Y translocations, and der(X) and der(Y) chromosomes with a range of p and q arm breakpoints on X and Y (Hsu, 1994). The phenotypes are male if *SRY* is present, and otherwise female. Infertility is typical, and, in the male, short stature. In the der(Y) case, in which there may be an effect of functional X disomy, genital anomaly and other malformation is common, as is mental defect. A detailed case is described in Ghosh et al. (2008), in which the recognition of an ultrasound brain anomaly at 21 weeks led to amniocentesis with the discovery of a de novo 46,X,der(Y)t(X;Y)(p22.13;q11.23). The Yqh region was replaced by Xp material, which thus existed in the functionally disomic state.

X Chromosome Deletion.

The possibility of an inherited X-autosome translocation should be checked by doing the mother's karyotype. Alternatively, it may transpire that the mother has the same karyotype. X chromosome deletions in the female, 46,X,del(Xp) or 46,X,del(Xq), predict the possibility, but not the certainty, of an incomplete form of Turner syndrome and/or premature ovarian failure (Fitzgerald et al., 1984; Veneman et al., 1991). Brown et al. (2001) describe a mother, of tall stature (5 feet 10 inches), having a prenatal diagnosis of del(X)(q22q26); she herself had the same karyotype, and "the parents took comfort in the observation that in the mother the deletion had no apparent phenotypic effect." A normal baby girl was born. Mother and daughter showed completely skewed X-inactivation, the abnormal X being consistently inactive.

In the male, the 46,Y,del(X) state would be nonviable for all but the very smallest deletions, and major abnormality would be probable for those pregnancies that might be viable.

X Chromosome Duplication.

De novo X chromosome duplications, 46,X,dup(X), in the female might have been thought to be of minimal effect (a partial XXX syndrome) due to selective inactivation of the abnormal X. This is sometimes but not necessarily the case, and abnormal phenotypes, often including genital defect, are not infrequently observed. Zhang et al. (1997) provide detail according to the extent and site of the duplication in a review of postnatally diagnosed cases. Tihy et al. (1999) describe an infant girl with a de novo dup(X)(q22.1q25) who had physical and neurodevelopmental defects, in spite of the X-inactivation pattern (at least on peripheral blood) showing consistent inactivation of the dup(X). Functional disomy, at least for part of the segment, may contribute to the abnormal phenotype in such cases (Armstrong et al., 2003; Tachdjian et al., 2004). Normality has been reported with respect to an isodicentric X, idic(X)(q27), comprising practically a double copy of the X, identified prenatally, the abnormal chromosome being late replicating, and indeed one such child was "academically advanced and enrolled in a gifted and talented program"; in contrast, some postnatally diagnosed patients have presented a Turner-like clinical picture (Tsai et al., 2006). In the male, functional disomy for the duplicated segment would likely cause severe defects, often lethal in utero.

The tiny marker X seen in the 45,X/46,X,+min(X) karyotype may be considered in the same category as the tiny ring X syndrome, discussed later.

Other Abnormal X Chromosomes

X chromosome abnormalities are characteristically seen in the mosaic state, the other cell line typically being 45,X. Mosaicism with a large ring X or an isochromosome for the X long arm, 45,X/46,r(X) and 45,X/46,X,i(Xq), respectively, would lead to variant Turner syndrome. An isochromosome for the X short arm, i(Xp), would probably always be

lethal, since there would be a functional Xp trisomy (Lebo et al., 1999). As with the small ring X syndrome (see following), a marker X that lacks *XIST* is associated with phenotypic abnormality (Tümer et al., 1998).

The “tiny ring X syndrome” with the karyotype 45,X/46,X,r(X) may have a functional X disomy and is typically, but not universally, seen with a severe phenotype of physical and mental defect, in some resembling Kabuki syndrome. The severity of the phenotype has been attributed to a functional X disomy, due to the ring lacking the *XIST* locus and thus not undergoing inactivation, although in rare instances this scenario may not apply (Migeon et al., 2000; Rodríguez et al., 2008). Similarly, in the male with the tiny ring as a supernumerary chromosome, usually as 46,XY/47,XY,+r(X) mosaicism, the clinical picture is typically abnormal, and in some severely so (Baker et al., 2010). Chen et al., (2006e) report an exception, from the prenatal diagnosis at amniocentesis of 46,XY[17]/47,XY,+mar[6], the marker turning out to be a very small *XIST*-negative r(X). The infant boy, on whose blood the proportions of the two cell lines were similar to the amniocentesis findings, was normal physically and developmentally, on follow-up to 1 year of age. These phenotypic differences may reflect the fraction and distribution of the abnormal chromosome.

Abnormal Y Chromosome

Hsu (1994) lists several possibilities: Yq– of various extents (excluding normal Yq variation), Yp–, r(Y), and isochromosomes or isodicentric chromosomes, written variously as i(Yp), idic(Yp), i(Yq), and idic(Yq). Concomitant 45,X mosaicism is very often observed, and this complicates prediction; ultrasonographic assessment of external genital morphology may be useful. Intactness of Yp with loss of Yq loci, and in particular the AZF spermatogenesis loci (Fig. 6–1), is associated with male infertility. Absence of *SRY* leads to female development, and loss of other Yp loci determines a Turner syndrome phenotype. In 45,X/46,X,der(Y) with Turner syndrome there is a risk for gonadoblastoma (Atkins et al., 2000). The outcomes in one prenatal diagnostic series are set out in Table 27–3.

Y Isochromosome.

The least rare of these rare conditions is the Y isochromosome, or isodicentric chromosome, usually seen as 46,X,i(Y)(p10) or 46,X,i(Y)(q11), in which the essential imbalance is a double dose of Yp material, and absence (or nearly so) of Yq.[11] As noted earlier, the condition may be seen in both nonmosaic and (more usually) mosaic form, the latter with a 45,X cell line. The phenotype in postnatally identified cases has ranged from sterile but otherwise normal male, through female somewhat resembling “testicular feminization,” to actual genital ambiguity (Bruyère et al., 2006; DesGroseilliers et al., 2006). In contrast, the outlook from unbiased (that is, not following an abnormal ultrasound) prenatal diagnosis is markedly in favor of normal male physical development, albeit that infertility will be very probable, and indeed, practically certain (p. 391). Ultrasonography is crucial: if this indicates male genitalia, a normal male phenotype is to be anticipated. Willis et al. (2006) reviewed 15 cases, with follow-up from 4 months to 9 years: all but one had presented as normal males, and “development has been normal in all cases where follow-up was reported.” A similarly optimistic interpretation comes from Bruyère et al. (2006): in a series of 12 cases from these authors, all 9 in which diagnosis had been unbiased, and the pregnancies continued, led to births of normal males, and normal development in those who were further followed up. While a question about cognitive development is not entirely settled[11] (Tuck-Muller et al., 1995; Neas et al., 2005), and few reports give follow-up into adolescence or adulthood, at least anecdotally, some do well.

Y Ring.

Layman et al. (2009) report their own cases and review the 45,X/46,X,r(Y) karyotype, as identified in males in whom testes were descended. Variable short stature and gonadal failure were typical. These authors note the confounding factors, in terms of predicting phenotype at prenatal diagnosis, of the bias toward genital abnormality in postnatally identified infants, versus the frequent lack of follow-up in apparently normal males following a prenatal diagnosis, leading to a bias in the other direction.

Three very rare Y scenarios may be mentioned. *(1)* The pericentric inversion Y is typically regarded as being a nonpathogenic variant (p. 173), although Gimelli et al. (2006) have reported a young *woman* with such an inversion, who presented with gonadal dysgenesis and bilateral gonadoblastoma. *(2)* A single case is recorded of a retarded man who had two copies of an idic(Y)(q12), conveying a tetrasomy of Yp; he rather resembled the picture of the XXXXY syndrome (Maas et al., 2005). *(3)* Mosaicism for a Yqh of different lengths, the shorter presumably having been derived from postzygotic deletion of the longer, has been identified prenatally in a very few cases (Cotter and Norton, 2005); a phenotypic consequence would seem unlikely, unless a Yq gonadal locus had been affected, which might theoretically compromise fertility.

Notes:

[1] An exception may be mosaicism for an isochromosome, as a handful of reports have demonstrated true mosaicism in the context of a single abnormal cell at prenatal diagnosis (see later).

[2] While we do not overlook the astonishing suggestion of Westra et al. (2008) that mosaicism in individual brain neurons may be a normal phenomenon, it is scarcely debatable that genomic imbalance due to a specific chromosomal defect, in a fraction of cells of the cerebral cortex, would in some wise affect intellectual functioning.

[3] CPM is the main, but not the only cause of discrepancy between the CVS and fetal/child karyotypes. One very rare explanation is that there was a resorbed co-twin with a different karyotype, with the sampling instrument having traversed its placental remnant (Tharapel et al. 1989).

[4] In this study, chromosomes 5, 8, 9, 10, 11, 12, 14, 15, and 16.

[5] Or an abnormal result from a simultaneous cord blood or amniocentesis, typically done in the context of abnormal fetal ultrasonography.

[6] As noted earlier, a few instances of apparent nonmosaic trisomy at CVS are also included here, on the assumption that—in the circumstance of a semblance of normal fetal development—a true fetal nonmosaic trisomy for that chromosome would in fact be improbable. We assume in these cases, rather, that this would be either “fetal mosaicism, nonmosaic placenta,” or “fetal-placental mosaicism” with the sampling needle missing the karyotypically normal tissue, each of these scenarios being demonstrated in Figure **27–4**.

[7] And yet, a new technology may, in its early days, return a question rather than an answer. Rooryck et al. (2010) found an apparently balanced de novo 2;18 translocation in a child with oculo-auriculo-vertebral syndrome and proceeded to a microarray analysis. This showed that each breakpoint was in a gene desert, and no nearby plausible candidate genes that might have been influenced due to a position effect; and furthermore, a microduplication elsewhere on chromosome 18 was identified, not recorded as a known CNV, but which was paternally inherited. What, if any, responsibility these genomic alterations had, severally or separately, for the genesis of the child's phenotype remains, for the moment, speculative. Had this analysis been done at prenatal diagnosis, the interpretation would have been fraught.

[8] The formally correct nomenclature is actually der(13;13)(q10;q10).

[9] Such an example comes from the prenatal diagnosis and postnatal confirmation of 47,XX,+mar/46,XX, the small marker of either 14 or 22 derivation, in which further studies indicated the probable scenario to have been a 47,XX,+22 conception, but then with correction brought about by conversion of one chromosome 22 into the SMC (along with maternal UPD22), in the lineage of the embryo. The infant showed normal/advanced early development (Bartels et al., 2003).

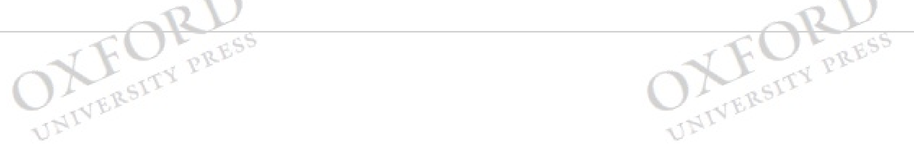
[10] Besides detection prenatally, the condition may be screened for at different stages of postnatal life. Herlihy et al. (2010) use Klinefelter syndrome as an exemplar of how the pros and cons of diagnosis versus nondiagnosis may be assessed, at different times of life.

[11] An interesting question, not entirely theoretical in the present context, is what extrapolation, if any, can be made from the XYY syndrome, in which there is a double dose of Yp, but of course also of Yq material. Neas et al. (2005) suggest that trisomy for the pseudoautosomal region PAR1 might lie behind aspects of the cognitive phenotype in the XXX and in some i(Y) karyotypes; and the same might apply to XYY.



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Gonadal Cytogenetic Damage from Exposure to Extrinsic Agents

Chapter: Gonadal Cytogenetic Damage from Exposure to Extrinsic Agents
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IN THIS CHAPTER we review what is known about the effects of some anticancer treatments, and of certain other therapeutic and environmental agents, that could conceivably have an injurious effect upon chromosomal distribution at gametogenesis, or which might cause chromosomal breakage or rearrangement in the cells of the gonad. In other words, the focus is on factors that might disturb the course of meiosis, or that might have clastogenic effects upon the chromosomes of gametocytes. We do not consider other categories of genetic damage.

Given the inherent vulnerability of gametogenesis, a logical starting position might have been that any potential damaging agent should be presumed guilty until proven innocent. As discussed in Chapter 23, large fractions of sperm and eggs, in the vicinities of 10%–20%, are chromosomally abnormal, due to aneuploidies or structural change acquired, for the most part, during meiosis. If this is what happens naturally, if gametogenesis is so susceptible normally, then surely would not agents known to compromise the integrity of the DNA and of the spindle apparatus (not to mention various artificial dietary and environmental exposures) compound the effect dramatically? Perhaps surprisingly, this seems not to be the case. Gametogenesis—provided the damage is not irreversible—often proceeds normally, or at any rate recovers, even in the setting of some heavy exposures, and no discernible increase in chromosomal abnormality is recorded in the subsequently born children. Nevertheless, if only on the pure grounds of what seems biologically reasonable and plausible, the question is not to be regarded as being closed. The fact that sperm chromosomes may, with certain agents, show an increased rate of cytogenetic abnormality is a more practical reason for maintaining a cautious view.

We outline the observations and conclusions relating to a number of medical conditions, cancer and otherwise, and touch on some environmental and lifestyle factors. The listing is not exhaustive.

Biology and Epidemiology

Cancer Treatment

A majority of children and young adults who receive modern cancer treatment survive. Some treatments cause sterility, but in quite a number fertility is unscathed, or at any rate subsequently recovers (Green et al., 2009, 2010). For those who are potentially capable of having children, the question arises: could there be an increased risk to have a child with a chromosomal abnormality? For most, in fact, the short answer may be, apparently not. Longer answers follow.

The chemotherapeutic agents and radiation used to rid the body of cancer are essentially cellular toxins, some of which specifically target DNA or the mitotic apparatus. Thus, the starting hypothesis is that the chromosomes in exposed bystander tissues, and thinking in particular of the gonad, could be vulnerable. The fact that these treatments can damage chromosomes is well known, and this is actually the basis of one of the in vitro laboratory tests for ataxia-telangiectasia.[1] Rapidly dividing cells are the most vulnerable to anticancer treatments (this being, of course, the rationale for their use). This would suggest, in theory, a susceptibility for spermatogenesis in the postpubertal male (millions of cell divisions daily), and a relative resistance in the prepubertal child (male meiosis yet to commence) and in oögenesis from infancy through menopause in the female (cell division in suspension). The direct assessment of gametic chromosomes offers insight. Sperm chromosome analysis can be done in men who have survived cancer treatment. Table 28–1 carries a review of 12 such studies and shows that several therapeutic regimens can cause sperm karyotypic defects.[2]

Table 28–1. Findings from 12 Sperm Chromosome Studies That Have Been Done in Respect of the Treatment of Certain Cancers, Showing Those Regimens That Are Associated, and Those That Are Not, with Sperm Chromosomal Abnormalities

			INCREASED FREQUENCY CHROMOSOME ABNORMALITIES	
CANCER	THERAPY	CYTOGENETICS	STRUCTURAL	NUMERICAL
Cancer	PEB and D-act and CY-CH-V-MT	Humster	+	+
Testic. ca. (non-sem.)	PVB, PVB and PEB	Humster	+	+
Rhabdomyosarcoma	CYVADIC	Humster	+	–
Ewing sarcoma	VAC	Humster	+	–
Wilms tumor	RT ± D-Act	Humster	+	–
Seminoma	PVB	Humster	–	–
Hodgkin's	MOPP, MOPP and RT	Humster	+	+
Lymphoma	MACOP-B	Humster	–	–
Lymphoma	MACOP-B	FISH	+	–
Embryonal cancer	PEB	FISH	–	+
Embryonal cancer	PEB	Humster	–	–
Hodgkin's	NOVP	FISH	+	+/–
Hodgkin's	Vinb. and RT	FISH	+	++
Testicular cancer (non-sem.)	PEB	FISH	+	++
Testicular cancer (non-sem.)	PEB	FISH	+	+

"humster" = pseudofertilization human-hamster test (p. 379). Testic. ca. (non-sem.) = testicular cancer, nonseminoma. Treatment regimens: CY-CH-V-MT, cyclophosphamide, chlorambucil, vinblastine, methotrexate; CYVADIC, cyclophosphamide, adriamycin, vincristine, dicarbazine; D-Act, D-actinomycin; MACOP-B, methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone; MOPP, nitrogen mustard, vincristine, procarbazine, prednisone; NOVP, novanthrone (mitoxantrone), vincristine, vinblastine, prednisone; PEB, cisplatin, etoposide, bleomycin; PVB, cisplatin, vinblastine, bleomycin; RT, radiotherapy; VAC, vincristine, adriamycin, cyclophosphamide; vinb., vinblastine.

Source: From the review of De Mas et al. (2001).

In practice, it is to the experience of the "therapeutic experiments" of oncological medicine that we mostly appeal: the in vivo observations of those who have survived their cancer, recovered from their treatment, and who have gone on to have, or to attempt to have, children. Have the children shown any excess of cytogenetic abnormality? The two largest studies are those from the Five Center[3] collaboration in the United States and a nationwide survey from Denmark (Byrne et al., 1998; Winther et al., 2004). In the American study, 1062 survivors born in the period 1945–1974 were interviewed in 1980–1983, these persons having had several types of childhood cancer (Hodgkin's disease, soft tissue sarcoma, and thyroid and central nervous system tumors accounting for the majority). In Denmark, a similar cohort of 4676 cancer survivors were compared with their 6441 siblings. The outcomes in the children were assessed. The rates of chromosome abnormalities in the offspring in these two studies did not differ significantly from those of their cancer-free siblings (Table 28–2).

Table 28–2. Outcomes from Two Large Studies of the Offspring of Childhood Cancer Survivors

	UNITED STATES		DENMARK	
	SURVIVORS	THEIR SIBLINGS	SURVIVORS	THEIR SIBLINGS
Numbers	1062	2032	4676	6441
Offspring	2198	4544	2630	5504
Chromosome abnormalities	0.2%	0.1%	0.21%	0.21%
Down syndrome*	0.1%	0.1%	1.07	1.0
Turner syndrome*			1.32	1.0

* U.S. figures = birth prevalence; Danish figures = relative risk. The gender of the affected parent of the DS and TS offspring in the Danish material suggested the nondisjunction event would more likely have been in the *un*affected partner, and thus the relative risks listed above (in any event statistically nonsignificant) may misrepresent a cancer-related effect. The American data are reevaluated in Meistrich and Byrne (2002).

Sources: From Byrne et al. (1998); Winther et al. (2004); and see text.

Specific Therapies

Chemotherapy.

Levy and Stillman (1991) and Arnon et al. (2001) review in detail the effects of various chemotherapeutic regimens upon fertility, in some of which data are also available concerning mutagenicity. The six classes of chemotherapeutic agents are the following: alkylating agents, cisplatin and its analogs, vinca alkaloids, antimetabolites, topoisomerase inhibitors, and "newer agents." In broad terms, the relationship between type of drug and risk for gonadal damage is outlined in Table 28–3.

Table 28–3. Three Categories of Risk for Germ Cell Depletion, Based on the Drugs Used

DEGREE OF RISK	DRUG
Definite	Mechlorethamine, chlorambucil, procarbazine, busulfan, cyclophosphamide
Probable	Doxorubicin, vinblastine, cytosine arabinoside
Unlikely	Methotrexate, 5-fluorouracil, 6-mercaptopurine

Source: From Levy and Stillman (1991).

Alkylating agents (cyclophosphamide and chlorambucil being major representatives), which have their damaging effect by adding an alkyl group to DNA, can, in the male, cause testicular hypotrophy, with oligospermia or azoöspermia. In follow-up into adulthood, the reproductive potential for males having been treated with an alkylating agent in childhood is considerably reduced, with a relative fertility of 0.4 (Byrne et al., 1987). Levy and Stillman review a number of papers, which offer a generally optimistic picture for girls in terms of pubertal development, but, as they point out, longer-term studies relating to the specific question of fertility are not so numerous. In one follow-up study, women actually had a relative fertility of 1.0 (Byrne et al., 1987). In adult women who have had chemotherapy with cyclophosphamide for Hodgkin's disease or breast cancer, oöcyte depletion and ovarian failure are documented (Familiari et al., 1993; Goodwin et al., 1999).

Antimetabolites, alkaloids, and antibiotics (including methotrexate, vincristine, actinomycin D) seem not to cause compromise ovarian function when given alone or as combination therapy, but in conjunction with radiotherapy some will cause ovarian failure.

Topoisomerase inhibitors affect the integrity of the mechanical apparatus of the meiotic chromosome, including the centromere and the microtubules of the spindle, and they also act directly upon the DNA; mouse studies with etoposide show an actual increase in sperm and zygote aneuploidies (De Mas et al., 2001; Marchetti et al., 2001).

Multiagent chemotherapy is, as would be expected, more damaging, and as illustrated in a group of girls who had succumbed to their cancer, from the observations of ovarian histology post mortem (Nicosia et al., 1985).

Radiotherapy.

Fertility is diminished in females who have had radiation therapy to the abdomen, and there is an increased risk of obstetric complication. But their children appear to have no increased incidence of birth defects (Nicholson and Byrne, 1993). Martin et al. (1986b) studied 13 male cancer patients (mostly seminoma) at intervals up to 36 months after radiotherapy, in whom the doses of testicular radiation were estimated to be in the range 0.4–5.0 Gray. While most were azoöspermic in the first year following treatment, in those in whom spermatogenesis recovered, variable increases in sperm chromosome abnormalities were seen, averaging two-fold overall compared with controls, but with wide ranges. The frequencies correlated with the estimated "bystander" testicular radiation (that is, the extent to which exposure extended beyond the target tissue).

Radioisotopes.

Radioiodine is used in thyroid cancer, and in a review of 408 offspring of survivors in Ehrenheim et al. (1997), no increase in congenital malformations was noted, although in one case a 7/14 translocation, not further described (and not identified as de novo or familial), was detected at prenatal diagnosis. One case does not make a case, but it can be noted.

Specific Diseases

Hodgkin's Lymphoma.

Treatment for Hodgkin's lymphoma typically involves radiation to the chest and abdomen, and multidrug chemotherapy (e. g., MOPP, NOVP, ABVD; see abbreviations in

footnote of Table 28–1). Spermatogenesis is compromised, and may or may not recover, although ovarian function typically is resistant or, if affected, more readily returns to normal (Marmor and Duyck, 1995; Papadakis et al., 1999). Some males have increased sperm aneuploidy rates before starting treatment, pointing to an effect upon spermatogenesis of the disease state itself. Tempest et al. (2008) review previous work on sperm chromosome analyses pre and post treatment, and report their own findings. In their material, chromosomes 13, 12, X, and Y were analyzed by fluorescence in situ hybridization (FISH), in very large numbers of sperm, from five patients age 19–36 years, and sampled at 0, 6, 12, and 18–24 months. Disomies and nullisomies for these chromosomes occurred at a higher frequency than in controls, albeit that the absolute amounts were small, at fractions of a percent. The aneuploidy rate declined to pretreatment levels by 18–24 months, although the authors retain a reservation that a 24-month cutoff could not necessarily be taken as a timeframe beyond which no increased risk would exist.

The foregoing work on sperm notwithstanding, actual observed reproductive outcomes from previously treated patients are the proof positive, and the observations are substantially reassuring. Aisner et al. (1993) interviewed 35 women and 25 men who had had 68 living children. There was no increase in spontaneous abortions or congenital abnormalities. Six women in the study of Papadakis et al. (1999) had had eight normal children. Similar findings were obtained by Swerdlow et al. (1996), from 11 men and 16 women who had had a total of 49 children. Chromosome analyses were done in 45 of these children, and all were normal, except for one child with trisomy 21, the additional chromosome having been transmitted from the other parent. These authors concluded that “offspring of patients treated in adulthood for Hodgkin's disease are not at greatly raised risk of genotoxic or other adverse outcomes as a consequence of their parent's treatment.” This provides a counterpoint to the concern raised by the in vitro sperm studies discussed earlier. It would be discretionary whether prenatal diagnosis might be chosen in a pregnancy conceived either by using pretreatment stored sperm or in a natural pregnancy following recovery of spermatogenesis.

Childhood Acute Lymphoblastic Leukemia.

About half of males treated for acute lymphoblastic leukemia (ALL) will suffer sterility, following the typical regimen of alkylating agents. López Andreu et al. (2000) undertook sperm analyses in a group of 22 childhood leukemia survivors, they at the time being teenagers or young adults. Five were azoöspermic or severely oligoasthenozoöspermic. The ovary, on the other hand, is more resistant to a permanent effect of alkylating agents. In those in whom fertility recovers, there has been no indication of any increase in the rates of congenital malformation in the offspring (Levy and Stillman, 1991). Kenney et al. (1996), in a study based on 140 children born to ALL survivors, record one case of trisomy 13, but overall there was no significant difference in malformation rates between the children of survivors (3.6%) and those of their sibling controls (3.5%).

Wilms Tumor.

In a review of Wilms tumor survivors, Byrne et al. (1998) documented a particular risk for the female to have children with adverse outcomes, but this effect is apparently due to a damaging influence of radiation on the uterus with a secondary deforming effect on the fetus, or possibly reflecting a transmitted genetic defect that had been responsible for the mother's original tumor as a child. No malformations were recorded in the offspring of 19 children of male survivors, this being, admittedly, a small number.

Testicular Cancer.

This is typically a cancer of younger men. The treatment is usually surgical removal of the affected testis, and chemotherapy with such agents as cisplatin, etoposide, and bleomycin. Fertility is maintained in some, although persisting oligospermia is a frequent observation, especially in those having had high-dose cisplatin (Stephenson et al., 1995). Sperm aneuploidy rates are increased, using certain chromosomes as surrogates, but the normality rate per autosome usually remaining above 99½%, and per sex chromosome, above 99%, in each case barely different from control data (Tempest et al., 2008). A risk to produce aneuploid sperm may diminish with the passage of time after the chemotherapy. Martin and colleagues (1997, 1998, 1999) studied four men variously before, during, and some 2–13 years after treatment with the three drugs noted. These men, after successful treatment, had six more children, all normal. Another eight normal children are recorded in the series of Stephenson et al. (1995), three of the fathers being oligospermic. While it appears that the risk for chromosome abnormality in a child is small, it may nevertheless be reasonable to offer prenatal diagnosis for fathers-to-be.

Seminoma.

No cytogenetic abnormalities were seen in the sperm of a 26-year-old man treated with cisplatin, vinblastine, and bleomycin, analyzed some 9 months after treatment (Jenderny et al., 1992).

Hydatidiform Mole.

This chromosomal condition is discussed in detail in Chapter 23. There is no increased risk for other abnormal outcome in a subsequent pregnancy, and in particular the incidence of congenital malformations is no greater (Berkowitz et al., 1994). Chemotherapy, either at the time of evacuation of complete mole, or for “persistent gestational trophoblastic tumor” following the index pregnancy, seems also to be without untoward effect in a subsequent pregnancy.

Other Disease.

Views differ whether there exists an increased risk for women with diabetes mellitus to have a child with Down syndrome (DS) (Narchi and Kulaylat, 1997; Martínez-Frías et al., 2002); it would be premature, as yet, to extrapolate the findings from a mouse model, in which oöcyte chromosomal misalignment was observed (Wang et al., 2009c).

Infertility Associated with Cancer Therapy, and Prior Gamete Banking

Preservation of gametes prior to treatment for cancer is a logical management, and sperm banking as “fertility insurance” for boys and men with cancer is now seen as routine (Menon et al., 2009). Blackhall et al. (2002) report 33 couples over a study period 1978–1990, the male being a survivor of Hodgkin's, using cryopreserved sperm for artificial insemination or IVF, with nine of the 33 having 10 pregnancies. One pregnancy was terminated due to fetal hydrocephalus (46,XX karyotype); the others all produced normal infants.

Cryopreservation of ovarian tissue is a more difficult procedure and still regarded as “investigational” (Tao and Del Valle, 2008). Any risk for chromosomal abnormalities in offspring is unknown, and data will need to be collected (it would be rather hypothetical to think that the risk in women might actually be reduced, if they were to reanimate their oöcytes from a younger age).

Immunosuppressive Treatment

Azathioprine is a major immunosuppresive agent, and its inimical effect upon the ovary known (McDermott and Powell, 1996). Reports of any possible effect upon offspring are few. One example is to be noted of a dysmorphic child with two de novo abnormalities, an interstitial deletion and an apparently balanced translocation, 46,XY,del(7)(q21);t(6;14)(q21;q12), whose mother had been treated with azathioprine and prednisone for *systemic lupus erythematosis*, although little weight can be put on a single case report (Ostrer et al., 1984). Jenderny et al. (1992) studied a 36-year-old man who had been treated for 4 years with these same two drugs for *chronic active hepatitis* and showed no significant differences in sperm chromosome distributions from controls. Alkylating agents are used in the treatment of *nephrotic syndrome*. In women who were treated in childhood with cyclophosphamide, fertility may be little compromised, with 17 out of 18 girls in one series going on to have normal menstruation and fertility (Watson et al., 1986). Pubertal boys, and those receiving higher doses of cyclophosphamide, are likely to become azoöspermic, whereas prepubertal boys appear to have greater gonadal resistance. The use of chlorambucil for this condition is also associated with a high likelihood of azoöspermia (Levy and Stillman, 1991).

Other Pharmaceutical Agents

We list a few medications in which cytogenetic gonadal/gametic studies (human or animal) have been done. The reader with a particular inquiry is directed to the Web site of the TERIS teratogen information system, albeit that the data relate largely to teratogenic, rather than mutagenic agents, at http://depts.washington.edu/terisweb/teris/index.html.

Antimicrobials.

Griseofulvin is a fungicide quite commonly used for the treatment of tinea. It is known to interfere with formation of microtubules, and it can damage the mitotic spindle. Mouse studies (although at very high dosages) suggest that meiosis may also be vulnerable, more so in oögenesis than in spermatogenesis (Shi et al., 1999). Single studies of mouse model sperm analyses suggest an increase in aneuploidies with exposure to *Pyrimethamine*, an antimalarial agent, and *Miconazole*, used in the treatment of candidiasis (Aydemir and Bilaloglu, 1996; Hassan, 1997). Whether the doses of the above agents typically used in humans might have practical reproductive implications is quite unknown.

Benzodiazapines.

Diazepam is used in psychiatry, and Baumgartner et al. (2001) studied patients having been on chronic high-dose treatment. Sperm disomy rates, for the chromosomes analyzed (X, Y, 13), were approximately two-fold those of controls (and sperm counts were reduced).

Sex Hormones.

Most studies point to no effect of previous use of the oral contraceptive pill, in terms of a subsequent risk to have a child with DS (Kallen, 1989). It is speculative whether there might be an additive effect from use of the pill together with cigarette smoking (Yang et al., 1999).

Folic Acid.

There was no change in prevalence of trisomy 21 in Canada comparing before and after the institution of folic acid fortification of flour in the late 1990s (Ray et al., 2003). In terms of taking periconceptual vitamin supplements, Botto et al. (2004) observed no significant difference in the frequency of the major trisomies of such pregnancies.

Diagnostic Radiology

A small effect may possibly exist for DS with respect to previous X-rays to the abdomen and pelvic area; that is to say, for X-rays in which the gonads may have been within, or not far off, the field of the film. In a study of 156 mothers and 149 fathers, in whose DS children the "nondisjunctional parent" could be identified (using Q-banding polymorphisms), a history of X-ray exposure was more often recorded in older fathers and in younger mothers (Strigini et al., 1990). The odds ratio for the whole group was 1.85, although the lower limit of the 95% confidence interval was 1.0. If such an effect truly exists in the younger mothers (and the statistics were borderline), it would seem that this slight influence becomes diluted as they get older, and the age effect comes to be predominant.

Nonmedical Exposures

Radioactivity.

The human germline may be relatively resistant to the damaging effects of radiation, compared with some animal models (Neel et al., 1990; Adriaens et al., 2009).[4] The atomic bomb blasts at Hiroshima and Nagasaki in 1945 were not followed by a statistically significant difference in the rate of chromosome abnormalities in children subsequently conceived, in a study commenced in 1967 (Awa et al., 1987; Neel and Schull, 1991; Nakamura, (2006). The study population comprised 8322 individuals born 1946–1972, age range at the time of study 12–38 years, one or both of whose parents were within 2000 meters of the hypocenter "ATB" (at the time of the bomb), alongside a contemporaneous local control group of 7976, who were either more than 2500 meters from the hypocenter or not present in the city. Sex chromosomal abnormalities were seen in 2.28 per 1000 in the former group and in 3.01 per 1000 of the latter. The only instance of autosomal trisomy was a 15-year-old with standard trisomy DS, whose father had been exposed at Hiroshima. (Given the structure of this study, deceased younger children and infants with autosomal trisomy were not included, although it is also to be noted that in separate analyses in Neel and Schull no significant correlation existed between parental exposure ATB and the frequency of stillbirth or congenital malformations.) More children of exposed parents had a small supernumerary abnormal chromosome than in the controls (5 cf. 2, a difference not specifically commented upon in Awa et al.). Of the balanced structural rearrangements, only two were confirmed as having arisen de novo (one each in the exposed and control groups). An earlier study with specific reference to clinically diagnosed DS in 9-month-old infants, undertaken during 1948–1954 (before the chromosomal basis of DS was known), had shown no increase among offspring of 5582 exposed cf. 9452 unexposed mothers, and indeed the figures were in the other direction (0.54 cf. 1.27 per 1000), and in spite of the exposed mothers being on average slightly older (Schull and Neel, 1962).

The Chernobyl nuclear plant explosion occurred in 1986, and a cloud of radioactivity was dispersed over Europe. With respect to DS, no subsequent rise in incidence was identified in a number of European countries, apart from small clusters in Berlin and Belarus, the latter of interest in that the peak was confined to 1 month, 9 months after the explosion (Little, 1993; Zatsepin et al., 2007). In contrast, Bound et al. (1995) suggest a possible link between events in 1957 (a fire in a nuclear reactor) and the early 1960s (increased levels of fallout from nuclear testing) and peaks of DS prevalence in 1958 and 1963–1964 in the Fylde district of Lancashire, England. But by no means is a firm case made: *post hoc* does not necessarily mean *propter hoc*,[5] and some fluctuation is normal. The same 1957 nuclear reactor accident had been proposed as the possible reason for a cluster of six cases of DS among the children of women who had attended the same high school in Dundalk, Ireland, during 1956–1957, when they would have been aged from 12 to 19. Of the 387 births to the former pupils from this period, the expectation would have been 0.69 children with DS. However, a stringent review of the evidence, and including molecular analyses that showed one case to have arisen post fertilization, led to the conclusion that, in fact, chance was the probable basis for the "cluster" (Dean et al., 2000).

While the germline, at least from the evidence outlined earlier, is apparently resistant, the same cannot be said for the bone marrow. Numerous studies on radiation exposure have shown that stable chromosome rearrangements may be induced, as measured on peripheral blood samples. Indeed, it is proposed that these changes can be used as reliable biomarkers of exposure. Populations in whom this effect has been seen include Russian nuclear plant workers, comparing those exposed to plutonium and those to gamma rays, from 1949–1989; New Zealand navy personnel who had served on ships during nuclear bomb testing in the Pacific Ocean in the late 1950s; American radiation technologists who had begun practicing from before 1950 (ages at the time of study 71–90 years); and even astronauts, unprotected by Earth's atmosphere from solar radiation (Durante et al., 2003; Hande et al., 2003; Sigurdson et al., 2008; Wahab et al., 2008). We are unaware of any evidence that individuals exposed in these ways might have acquired any gonadal damage, and that their children could have been at risk for a chromosomal disorder. It would be a massive logistic exercise, but not without interest, if a study could be mounted of descendants of these exposed persons.

Industrial Agents.

Paternal occupation provides a surrogate marker for a variety of potential industrial agents. Olshan et al. (1989) assessed the father's occupation for 1000 DS children born in British Columbia in 1952 through 1973. Seven employment categories out of 59 showed odds ratios in the range 1.4–3.3, the lower confidence limit being not less than 0.9, in certain of which exposure to various industrial agents could plausibly have occurred (including mechanics, janitors, metal workers, sawmillers). But the increases in risk were small, and with 59 items there was of course the possibility of chance fluctuation. One category that might have seemed risky, namely "other chemical workers," in fact had the lowest odds ratio of all (0.2).

Pesticides have biological activity, and it is reasonable to raise a case that distribution across the blood-testis barrier might follow inhalation, or absorption, or ingestion, and that the local effect upon gonadal tissue might be toxic. Perry (2008) has reviewed 30 studies, correlating pesticide exposure with sperm chromosomal abnormality. The methodologies varied, so direct comparisons could not readily be made. Some studies did, and some did not, show an increase in chromosomal defects. Of the numerous

agents, the strongest case could be made for carbaryl and fenvalerate, in particular, as potentially causative of autosomal and gonosomal aneuploidy, with sex chromosome disomy the most frequent single abnormality.

The air we breathe, it is suggested, might convey mutagens, in the form of industrial pollutants, and these might reach gonadal tissue (Somers and Cooper, 2009). Landfill sites contain toxic matter, which might in theory contaminate the air in nearby residential areas; but in an analysis based upon over 6000 such sites throughout the United Kingdom, and comparing populations living within, and beyond 2 kilometers of these sites, in fact no differences in the prevalence of Down syndrome were observed (Jarup et al., 2007). However, older mothers living within one mile of industrial sites from which solvent and heavy metal emissions are vented may have a slightly increased risk for aneuploidies in their offspring (Brender et al., 2008); in the male, variation in air pollution may affect some aspects of sperm quality, although with no obvious influence upon disomy or diploidy rates (Rubes et al., 2005). Confirmatory studies are needed.

Bisphenol A.

Bisphenol A (BPA), an estrogenic chemical used widely in plastic manufacture, has been shown to disrupt several different stages of oöcyte development in mice (Susiarjo et al., 2007). The fetal ovaries observed after pregnant mice were treated with low, environmentally relevant doses of BPA during mid-gestation showed synaptic defects and increased levels of recombination. The mature females, exposed as fetuses, went on to have oöcytes and embryos with aneuploidies. There may be further environmental influences on the effect of BPA in that variations in diet influenced the observation of meiotic abnormalities in exposed mice (Muhlhauser et al., 2009), thus demonstrating the complexities in studying environmental exposures, since even in laboratory animals it is nearly impossible to keep all other variables constant. Reservations are already held concerning its use in human activity for other health-related reasons, and the mouse data might be seen as one further reason for caution.

Agent Orange.

Agent Orange (a mixture of phenoxylic herbicides) was used in the Vietnam War as a defoliant spray, and those exposed may have absorbed the chemical via the oral route in particular. A study of New Zealand Vietnam veterans some 3–4 decades after the war showed an increase in sister chromatid exchanges on blood samples (Rowland et al., 2007). Whether gonadal genetic damage results is controversial (Ngo et al., 2006; Schecter and Constable, 2006; Fraser, 2009); specifically, we are unaware of any evidence for an increased risk of chromosomal abnormalities in offspring.

Tobacco, Alcohol, Caffeine.

Tobacco smoking in mothers had no influence upon the incidence of DS in the study of Chen et al. (1999a), based upon data of a population case-control analysis in Washington state from 1984–1994, and in which they had been at pains to account for a confounding effect of maternal age. The odds ratio was exactly 1.0—that is, no effect either way—for smokers versus nonsmokers. Similar findings are also reported from Sweden, California, and England (Kallen, 1997; Torfs and Christianson, 2000; Rudnicka et al., 2002). Nevertheless, a tentative role has been proposed for one particular mechanism: trisomy 21 due to nondisjunction in maternal meiosis II (MMII). In a case-control study in Atlanta, cigarette smoking around the time of conception gave an odds ratio of 7.6 in mothers of MMII trisomic offspring, compared with controls, in the <35-year age group (Yang et al., 1999). Very speculatively, smoking might diminish blood flow in the microvasculature of the perifollicular bed, and the resultant hypoxia could compromise some aspect of the oöcyte's functioning as the meiotic process is reactivated. *Alcohol and coffee* taken by the mother prior to conceiving might actually reduce the DS risk. In the study of Torfs and Christianson, the odds ratios for "high" alcohol and coffee consumption (≥4 drinks per week, ≥4 cups per day) were 0.54 and 0.63, respectively. If these figures reflect biological reality, a possible mechanism would be a selective reduction in viability of a trisomic 21, as compared to a normal conceptus.

Concerning spermatogenesis, Shi and Martin (2000b), reviewing the literature, concluded that personal habits with respect to smoking, alcohol, and caffeine ingestion appear not to have any consistent effect upon disomy rates in sperm, although since there were somewhat varying findings in the different studies, it had to be acknowledged that a definitive answer was not at hand. Shi et al. (2001a) proceeded to a study of cigarette smoking and aneuploidy using FISH analysis of sperm, with reference to chromosomes 13, 21, X and Y. They divided their subjects into nonsmokers, light smokers (<20 cigarettes/day), and heavy smokers (≥20/day). The smokers showed an increase in disomic 13 sperm (0.2% of sperm 24,+13, versus 0.07% in controls), which was statistically significant. The rates of disomies 21, X and Y were within the control ranges. Chromosome 13 and, from other studies, chromosome 1 may be more susceptible, as they go through meiotic disjunction, to an untoward influence of toxic substances in cigarette smoke. Since most trisomy 13 is due to a maternal meiotic error, and given that the excess is, in absolute terms, very small, it seems safe to suppose that fathers who smoke contribute scarcely, if at all, to the totality of this particular aneuploidy. As for alcohol, the observation of a negative association between sperm disomy frequencies and alcohol consumption in one study[6] (Härkönen et al., 1999), and noting also the figures earlier on maternal consumption, should not lead one to advise that couples drink more heavily prior to a planned impregnation!

Second-hand smoke is difficult to assess, outside of controlled animal studies (Hung et al., 2009). A study of four adult male rhesus macaques, exposed to second-hand smoke for 6 months, showed no change in the X:Y ratio in sperm, which may indicate that there is no increase in aneuploidy. In addition, second-hand smoke-exposed pregnancies did not show increased DNA damage in their offspring, as compared to babies born to nonsmoking mothers (de Assis et al., 2009).

Genetic Counseling

As Wyrobek and Adler (1996) commented, "it has been more than half a century [1927] since Muller demonstrated that X-rays can induce germinal mutations in Drosophila, yet questions as basic as the existence of even a single human germinal mutagen remain unresolved." McFadden and Friedman, writing in 1997, noted that no environmental agent has been identified in which it could be stated, beyond reasonable doubt, that this agent would cause chromosome abnormalities in the offspring of exposed parents. While some studies have shown increased rates of aneuploidy in sperm, the practical fact remains that there is no excess in children born with chromosomal syndromes. Only in 2001 could Marchetti et al. claim, with respect to their work on etoposide exposure with a mouse model, that "we know of no other report of an agent for which paternal exposure leads to an increased incidence of aneuploidy in the offspring."

Encouragement can be drawn from this largely negative information, and the counselor will usually be justified in offering substantially reassuring advice from the particular focus of chromosome abnormality. Reference to the commentaries earlier may provide useful supporting information for the individual agent of specific interest. Prenatal diagnosis would be a discretionary option, as would be preimplantation diagnosis for those whose treatment-related infertility required IVF. A propos the specific question of previous treatment for cancer, Byrne (1999) does remain cautious, considering that on the rather few data available there are only "limited grounds for reassurance," and seeing us as being "in the infancy of studies of germ cell mutagenesis in cancer survivors." She emphasizes that the opportunity to assess newer cancer treatments has not yet arisen, and that the timeframe for these assessments is to be measured in decades. Certainly, and more particularly for those agents in which a biological link could plausibly be proposed, it is right that research in this area continue.

Notes:

[1] Radiation and bleomycin, both having potent DNA-breaking properties, cause lymphocyte chromosome rearrangements in normal, and considerably more so in ataxia-telangiectasia cells.

[2] Male cancer patients may show abnormal sperm genetic studies ahead of having received any treatment, suggesting that there is a harmful effect of the malignant disease per se (Tempest et al., 2008).

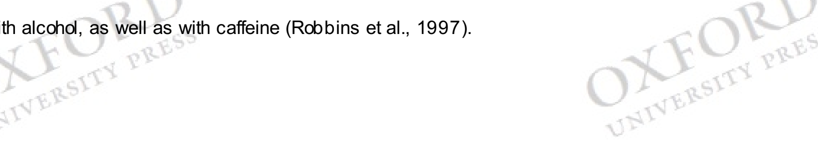
[3] Universities of Iowa, Kansas, and Texas, California Department of Health Services, Connecticut Tumor Registry.

[4] Of historic interest, a very early example of ill health due to radiation exposure is that of Marie Curie, who was awarded the Nobel Prize twice. One daughter of hers was a

scientist, and she also won a Nobel Prize, and the other was a skilled pianist and gifted writer. A series of n = 2 is very small, but rather evidently there must have been normal chromosomal segregation in the meioses leading to these two daughters.

[5] *Post hoc, ergo propter hoc* (Latin) = Something happened after the event, and therefore it must have been due to the event.

[6] But another study showed a positive association with alcohol, as well as with caffeine (Robbins et al., 1997).





Oxford Medicine









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